JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXIX WASHINGTON, D. C., November 1, 1924 No. 9

ALTERNARIA LEAFSPOT AND BROWNROT OF CAULIFLOWER [1]

BY J. L. WEIMER

Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In 1918 the writer's attention was called to a disease of cauliflower [2] which was causing considerable damage during transportation to the market. Inspectors for the Bureau of Agricultural Economics reported it frequently from the Chicago and New York markets on cauliflower from California and the Northwest. The disease probably developed in transit, since heads said to be healthy when placed in the cars were badly diseased on reaching their destination. From 10 days to 2 weeks usually elapse between the cutting of the cauliflower on the Pacific coast and its arrival in the eastern markets. This probably affords ample time for the disease to develop. However, there was no information available regarding the cause of this disease, its origin, or the conditions under which it developed. The investigations discussed in this paper were undertaken with a view to furnishing such information.

HOSTS

Although the brownrot as such occurs on only the heads of cauliflower, a leafspot caused by the same fungus occurs on a number of cruciferous plants. The leafspot on cabbage is especially well known. The writer has been able to obtain infection of young seedlings of kohlrabi, Brussels sprouts, broccoli, cabbage, and cauliflower in the greenhouse. An attempt to infect white mustard seedlings failed. A number of other closely related plants are also hosts for this disease.

NAME OF THE DISEASE

The leafspot is usually referred to as "Alternaria" leafspot but is sometimes called "black" leafspot and "blackmold" The term "brownrot" has been applied for the past several years to certain brownish-colored lesions of unknown origin commonly found on the curds of cauliflower on the market. Although these lesions do not all have a common origin, it is thought that the disease here discussed is their most common cause. The term "brownrot" is fairly descriptive of the disease, and since it is already in use by tradesmen it will be retained here. But care should be taken not to confuse this disease with other rots of the curd of this host which may also have a brown appearance. One such rot which is especially prevalent and which is most likely to be confused with the disease under consideration is of bacterial origin and is known by the trade as "slimy softrot." The term "brownrot" was applied in the past to a disease caused by *Bacterium campestre* (Pammel) Erw. Sm., but the name "black rot" is now used exclusively to designate this trouble.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Alternaria leafspot has a very wide distribution, both here and abroad. The disease on the curd of cauliflower is probably coextensive with the crop but is more destructive when the host is shipped long distances or is held for a considerable period under conditions favorable for its development. Puttemans (*13*)[3] in 1911 described what no doubt was this same disease occurring on curds of cauliflower found on the markets of Rio de Janeiro. He studied the disease somewhat and decided that it was caused by *Alternaria brassicae* (Berk.) Sacc. The disease was found to develop in transit during warm moist weather, notwithstanding the fact that the time consumed in packing and shipping the cauliflower was not longer than three to four days. The writer's data show that this rapid development of the disease may be expected at high temperatures and humidities.

[1] Received for publication April 10, 1924—issued January, 1925.

[2] The term "cauliflower" as here used also includes broccoli.

[3] Reference is made by number (italic) to "Literature cited," pp. 441-442

Fawcett (*8*) states that *A. brassicae* causes much damage to cabbage in Florida some seasons. Higgins (*11*) likewise reports this disease as having been very destructive on collards in Georgia for several years. Harter and Jones (*9*) state that black leafspot causes considerable damage to cabbage and collards in this country and in Europe. Chupp (*3*) records the fact that on Long Island in the cabbage-seed growing district Alternaria always does injury as a seed-pod spot. The literature, both domestic and foreign, is replete with references to Alternaria leafspot on cabbage and other cruciferous hosts. Notwithstanding its prevalence, the disease is usually of minor importance on cabbage in the field, since it ordinarly occurs only on parts of the host which have become low in vitality. With the exception of that by Puttemans (*13*), no reference to the occurrence of this disease on the curd of cauliflower has been found. No doubt the brownrot has been prevalent for a long time without having received much attention from scientists, due in part at least to the fact that until recent years little time has been given to the study of transit diseases. As stated above, the disease of the curds seems to be most prevalent on cauliflower from the Pacific coast. This does not mean that it is necessarily more common or destructive there. The writer has sought many times for this disease on the heads of cauliflower in the vegetable-growing section about San Francisco during the season of 1922–23 but never found it except on heads that were overmature or that had been cut from the plants and left lying on the ground. The growers in the Los Angeles section claim that this disease appears during the latter part of their shipping season. While the leafspot stage was not common in the San Francisco section it was very prevalent about Los Angeles during the season of 1922–23. Probably cauliflower from no other region is subjected to conditions so favorable for the development of the disease as is that from the Pacific coast during the period of transit across the continent to the eastern markets. Nevertheless, the writer has seen the typical brownrot lesions on cauliflower from New York. Badly diseased heads have also been obtained from the Washington, D. C., city market. Some of these were locally grown while others were said to have come from Florida. There is little doubt that the brownrot may be found wherever cauliflower is grown, provided it has been subjected to proper environmental conditions. But it appears to be of little importance except as a transit trouble.

A few figures from the reports of the Bureau of Agricultural Economics indicate some of the losses caused by this disease in transit (Table I).

Except under conditions of high humidity, the disease develops slowly and causes greater loss by badly disfiguring the leaves and heads than by actually destroying them. Under field conditions the leaves may be so badly affected that the vitality of the plants is lowered and the size of the heads consequently reduced. Other microorganisms such as soft-rot bacteria and species of Fusarium and Rhizopus also enter the lesions and initiate other types of decay.

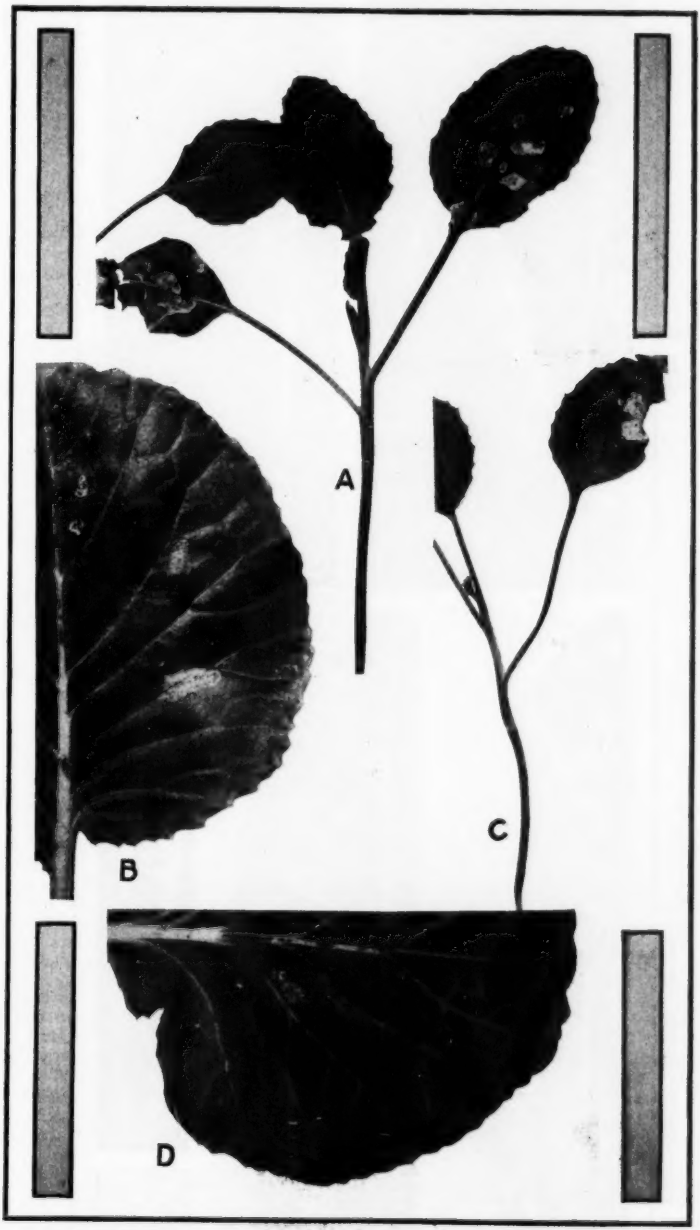
SYMPTOMS OF THE DISEASE

In the writer's experiments the disease first became evident on the leaves, petioles, and stems of young plants as small dark brown to almost black spots about 1 mm. in diameter. The ultimate size and character of the lesions depended to some extent on the humidity and on the nature of the tissue affected. Three types of lesions usually developed on leaves of cauliflower inoculated in the greenhouse (Pl. 1 and 2). On the lamina the spots remained small (about 1 mm.) and dark brown, developing but little after the first three days (Pls. 2, *b*), or they enlarged, forming lesions somewhat circular in outline from 0.5 to 1 cm. or more in diameter and grayish to brown in color. Scattered promiscuously about the center of these spots were minute black areas where the fungus was fruiting. These spots sometimes coalesced, forming large irregular areas, often involving half or more of the leaf (Pl. 1, A, C; Pl. 2, A, B, C, D). The diseased tissue shaded off from brownish to yellow and then to the normal green of healthy

EXPLANATORY LEGEND FOR PLATE 1

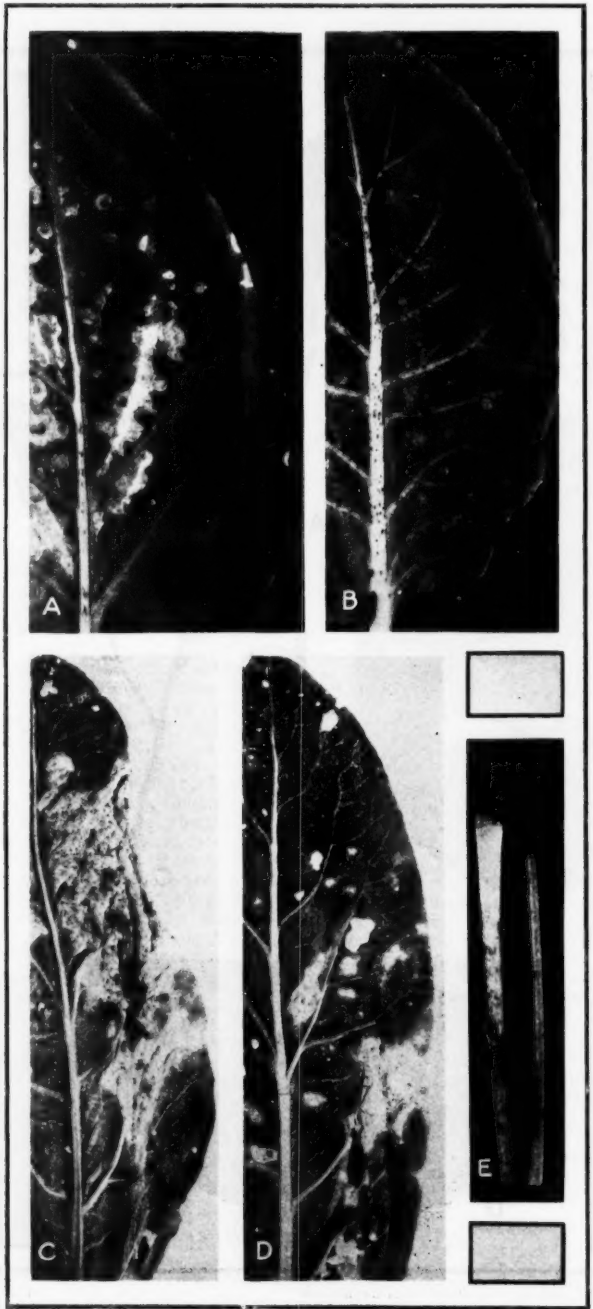
A and C.—Early Snowball cauliflower seedlings grown in the greenhouse, showing the type of lesions produced by *Alternaria brassicae* on the leaves and petioles. Photographed 3 days after inoculation. Natural size

B and D.—Seedling cabbage leaves grown in the greenhouse, showing the circular to irregular, light-colored lesions caused by *A. brassicae* as well as the small, dark-colored ones, the latter being limited largely to the veins. About natural size



Alternaria Leafspot and Brownrot of Cauliflower Plate 1

(For explanatory legend see p. 422)



Alternaria Leafspot and Brownrot of Cauliflower Plate 2

(For explanatory legend see p. 425)

tissue. Lesions of the third type resembled those of the first, except that they appeared on the larger veins, petioles, and stalks, and were linear in shape, having the longer diameter parallel to the veins, petioles, or stems. Under conditions of high humidity these lesions sometimes developed rapidly, so that the parts attacked were soon decayed through. Badly affected leaves finally turned yellow and dropped off. The area of leaf tissue which might have functioned in photosynthesis was greatly reduced and the plants failed to develop properly. Stems of seedlings were frequently weakened by the cankers produced by this disease, so that they broke over and died. Under conditions of high humidity the dead areas of the leaves were covered with hyphae and spores which gave them an olivaceous color.

In cauliflower leaves inoculated in the field (Pl. 2, C, D), large areas of leaf tissues were killed. This dead tissue was papery in texture and bore black spore masses. The high humidity and temperature at the time of inoculation and for several days thereafter no doubt account for the severity of the infection. The symptoms of the disease on cabbage leaves are for the most part the same as those on cauliflower. Plate 1, B and D, shows the different types of lesions on young cabbage leaves inoculated in the greenhouse.

This disease causes a browning of the individual buds or groups of buds of the curd. Plate 3, B, shows a very early stage of the disease on the curds, resulting from 5-day-old artificial inoculation. The color of the affected tissues varies with age as well as with the conditions of temperature and humidity. The infected blossoms are at first light brown, darkening with age to nearly chestnut brown, which is usually followed by an olivaceous color, due to the development of dark-colored aerial hyphae and spores. Plate 3, C, shows the same head illustrated in Plate 3, B, photographed 13 days after inoculation. The diseased spots had enlarged considerably and were of the typical olivaceous color. A similar condition is shown in Plate 3, D, which is a photograph of a naturally infected cauliflower head obtained from the market. Plate 4 shows a head which was inoculated in the field. Its flower stalks are elongated preparatory to the formation of seed. The blackened tissue was covered with spores and hyphae.

Lesions caused by this disease may be easily confused, especially in their early stages, with bruises and with soft-rot produced by bacteria. Microscopical examination is often necessary to determine the true nature of some of the small lesions. As the decay induced by bacteria develops, the tissue becomes soft, wet, and usually slimy. The tissue rotted by bacteria is light brown and has a water-soaked appearance.

ETIOLOGY OF THE DISEASE

In 1918, when this work was begun, the etiology of the disease on the curd of cauliflower was unknown, at least as far as could be ascertained from the literature. Isolations were made from a large number of affected curds of cauliflower from as many sources as possible and a pure culture of a species of Alternaria was almost always obtained. Careful study of this fungus has shown it to be the same as the one that causes the leafspot of cabbage and related plants, namely, *Alternaria brassicae* (Berk.) Sacc. This fungus was first described by Berkeley as *Macrosporium brassicae* (*1, p. 339*), but was later placed in the genus Alternaria by Saccardo (*14, p. 546*).

PATHOGENICITY

Although from brownrot lesions a single species of Alternaria was almost invariably obtained, sometimes a species of Fusarium, bacteria, or other species of Alternaria appeared in the plates. Inoculation experiments showed, however, that the species of Alternaria so commonly present in the lesions was the causal organism.

The method used in all of the experiments conducted to prove the pathogenicity of the strain of Alternaria isolated from the typical brownrot lesions was as follows: The preliminary tests were made in the greenhouse. Plants of different ages were sprayed

EXPLANATORY LEGEND FOR PLATE 2

A, B, and E.—Cauliflower leaves and petioles from greenhouse-grown plants, inoculated with *Alternaria brassicae* showing the circular, light-colored lesions which often coalesce, killing large areas of the leaf and also the small, dark spots (leaf B), which may spread but little. These leaves were sprayed with the same spore suspension at the same time and were held under identical conditions after being inoculated. Photographed 3 days after inoculation. *Alternaria brassicae* was recovered from both types of lesions. About three-fourths natural size

D.—Leaves of a mature field-grown cauliflower plant sprayed with a suspension of spores of *A. brassicae* during a hot, rainy period. The large, light-colored areas of the leaves were killed by the fungus. About one-half natural size. Photographed 6 days after inoculation

with a suspension of spores in sterile distilled water and then placed in a large moist chamber for about 48 hours. The leaves were first rubbed gently to remove the waxy bloom so that the spore suspension would stick. Likewise, the heads of cabbage and the curds of cauliflower were sprayed with a suspension of spores and then placed in moist chambers, usually battery jars lined with wet filter paper, and incubated at different temperatures. Heads and leaves of cabbage and cauliflower growing in the field were sprayed with a spore suspension and then the outer leaves were pulled up and tied about the head. Controls which consisted of plants from the same lot, treated in every way the same as those inoculated except that spores were not permitted to come in contact with them, were held in each experiment.

Usually isolations were made from the lesions formed on the inoculated host parts. In some instances a microscopical study was made which showed the typical Alternaria spores to be present in great abundance on the lesions. The organism isolated was shown to be capable of infecting a healthy host and producing the typical symptoms.

The results of several of the inoculation experiments are tabulated in Table II. A high percentage of infection was obtained in all cases. The number of spots produced on the heads and leaves varied from a few to very many, depending largely on the humidity and on the concentration of the spore suspension used. For the most part the leaves seemed more susceptible than the heads under field conditions, although a large number of infections on the latter were obtained. No injuries of any kind were made intentionally or could be observed on the curds of the cauliflowers before inoculation.

The experiments listed in Table II, together with those given in connection with temperature studies later, show conclusively that the species of Alternaria commonly associated with the leafspot and brownrot lesions is capable of producing such lesions under the conditions existing in these experiments. Other species of Alternaria were frequently found growing saprophytically on decaying cauliflower and cabbage. The fungi numbered 5089, 5095, and 4866a in Table II proved to be of this nature. Not only did these prove to be nonparasitic but they differed morphologically from the parasitic forms, all of which were alike.

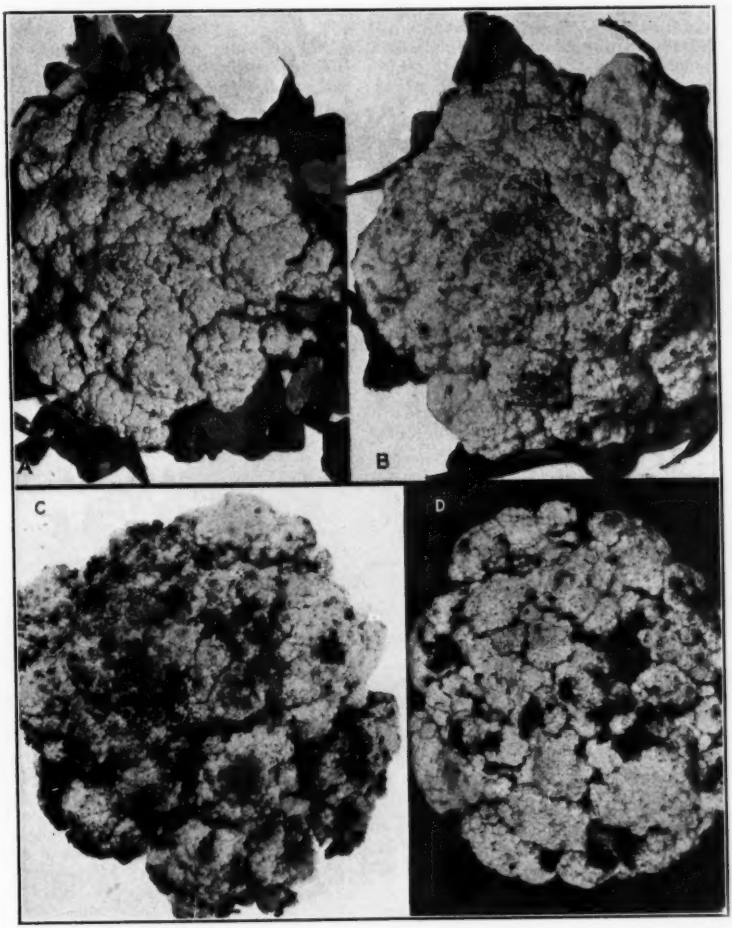
On the plants in the greenhouse which were inoculated on February 22, 1922, infection was evident as very minute dark-brown spots just visible to the unaided eye in 48 hours, when they were removed from the moist chamber. These spots were so numerous on some leaves that two-thirds or more of the entire leaf surface was involved. When examined 24 hours later, the large greenish water-soaked type of lesion was present and large areas of the leaves were killed. A similar condition is illustrated in Plate 2, C. The dead tissues later turned light brown in color and had a papery texture. Five days after the inoculations were made some of the petioles were so badly decayed that they broke off. Likewise, the laminae of some of the leaves were so severely affected that the leaves dropped off. So large a number of infections probably seldom, if ever, occurs on a host under natural conditions, yet one-half to two-thirds of the laminae of several of the leaves of cauliflower growing in the field which were inoculated on June 18, 1921, were dead six days later. Also the curds of these plants had large brownrot lesions at that time.

INFLUENCE OF TEMPERATURE ON SPORE GERMINATION AND MYCELIAL GROWTH

It has been pointed out that brownrot causes considerable damage to cauliflower while in transit. Cauliflower reported to be free from disease when placed in the car for shipment has often been found badly spotted on reaching its destination. The question naturally arises whether or not the fungus can develop at temperatures existing in the refrigerator cars in which cauliflowers are shipped. In order to determine this point, the effect of temperature on the rate of germination of the spores and the subsequent growth of the mycelium was studied. The method used was essentially the same as that described by Weimer and Harter (*18*). Preliminary tests showed that the spores germinated better in sweet-potato decoction (500 gm. sweet potatoes per liter) than in either tap or distilled water. Hence this medium was used.

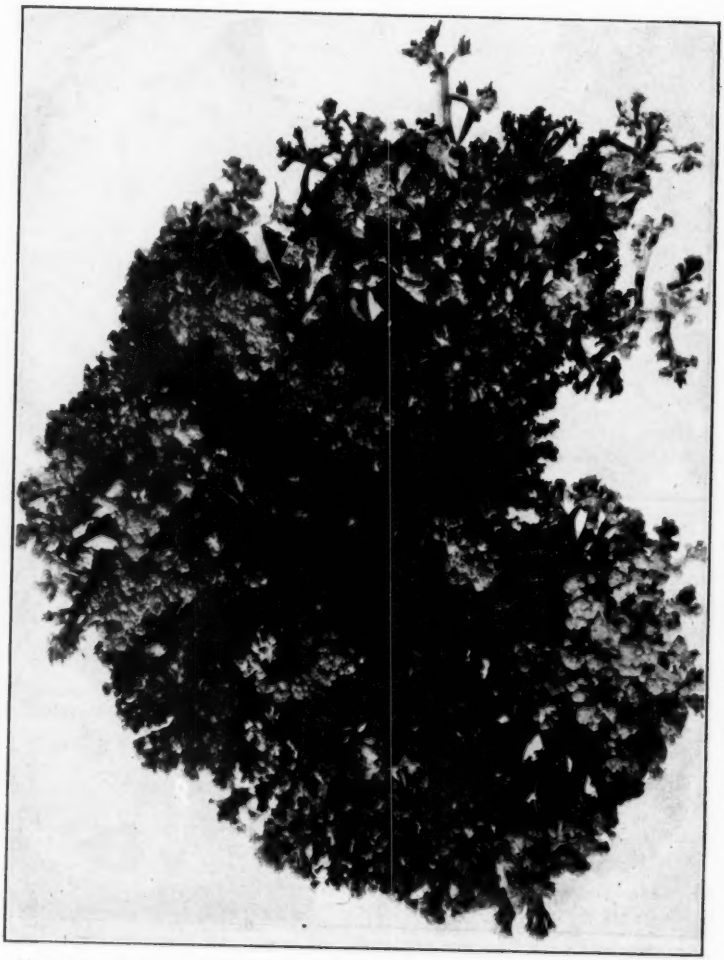
EXPLANATORY LEGEND FOR PLATE 3

A.—Healthy head of cauliflower held as control
B.—Cauliflower head showing small brownrot lesions resulting from inoculation by spraying with a suspension of *A. brassicae* spores from a pure culture. Photographed 5 days after inoculation. Natural size
C.—Same cauliflower head photographed 13 days after inoculation. Natural size
D.—Cauliflower head showing natural infection with *A. brassicae*. Lesions appear as black spots



Alternaria Leafspot and Brownrot of Cauliflower Plate 3

(For explanatory legend see p. 426)



Alternaria Leafspot and Brownrot of Cauliflower

Plate 4

(For explanatory legend see p. 429)

Loops of a spore suspension of the desired concentration were placed upon clean cover slips, which were then inverted over glass rings cemented to slides with a mixture of beeswax and vaseline, the cover slips being sealed to the rings with vaseline. A small quantity of the same medium used in preparing the spore suspension was placed in the bottom of each cell. The hanging drops were placed in the electrically heated and controlled Altman incubators as soon as possible after they were prepared. The temperature of these incubators remained quite constant, varying for the most part less than a degree, except for the lower ones, which depended upon the amount of ice in the cooling chamber, where there was sometimes a variation of 2° to 2.5° C. A pan of water was kept in the bottom of each chamber to keep the air moist. From four to eight hanging drops were used at each temperature. These were removed from the incubators at frequent intervals and observed under the microscope. The mounts were examined near the incubators, and were kept out usually less than a minute. Only at the highest temperatures was there any appreciable fluctuation due to the opening of the doors, and in these cases the readjustment occurred very quickly. In the different trials the average time found necessary for the spores which germinated first to produce germ tubes equal in length to the width of the spore was used as the time required for germination. The figures thus obtained, together with the average of the temperatures to which the spores were exposed in the different experiments, were used in plotting a curve which shows the variation in the time for germination to start due to the difference in temperature (fig. 1).

Other criteria might have been used for determining the influence of temperature upon this vital phenomenon. However, the important thing in connection with the study of brownrot is to know how soon infection can take place at the different temperatures and this obviously depends primarily upon the time necessary for the spores to germinate. In fact, it is usually of little practical importance to know whether or not all the spores germinate within a certain time at a given temperature since most fungi produce spores so profusely that if only a small percentage of them germinate a large amount of damage may result. About 99 per cent of the spores studied in these investigations germinated, except near the maximum temperature, where some of them were no doubt killed before germination started.

In the curve shown in Figure 1 the time in hours necessary for germination to take place is plotted on the abscissa, while the temperature in degress centigrade is plotted on the ordinate. The hanging drops were kept under observation for some time after germination started, to make sure that the other spores germinated soon afterwards. No germination took place at the maximum temperature tried (46° C.). As no temperature between 40.5° and 46° was tried, the temperature at which germination would have just taken place was not determined.

An examination of the curve shows that the optimum temperature for spore germination is somewhere between 33° and 35° C. From the optimum the time necessary for germination to begin gradually increased as the temperature became lower, but increased quite rapidly above this temperature until the maximum was reached. The minimum for germination was not determined accurately for want of a sufficiently low temperature. Germination took place in 48 hours at an average temperature of 1.5°. It is apparent from these data that so far as spore germination is a factor, infection by the brownrot fungus can take place in transit.

The effect of temperature upon the growth of the mycelium was studied in Petri dish cultures containing 20 cc. of a 2 per cent Irish potato agar. A small drop of a suspension of the spores in water was placed in the center of each plate with a 2 mm. platinum loop. The plates were placed in the incubators as soon as they were inoculated and the rate of growth determined by measuring twice daily the diameter of the mycelial disks formed. Five Petri dishes were used for each temperature. The experiment was repeated and the average diameters of the mycelial colonies as well as the average temperatures for the times that the cultures were grown were used in plotting the graphs in Figure 2. The base line of these

EXPLANATORY LEGEND FOR PLATE 4

Cauliflower head grown in the field, badly affected with brownrot resulting from artificial inoculation. This head has spread and the flower stalks have begun to elongate preparatory to blossoming. All of the blackened area was affected. About natural size

graphs shows the temperature in degrees centigrade; the perpendicular shows the diameter of the mycelial disks. The first graph shows the diameter of the mycelial disks at different temperatures at the end of 2 days, while the other graphs show their comparative diameters after 4, 6, 8, 10, and 15 days, respectively.

same throughout the experiment. No growth took place at 30.5° during the first two days, although a fair growth was made at 30°. There was some visible growth in four days at 33.5°, but none until the eighth day at the maximum temperature, at which a visible growth took place (36°). There was no growth even after 15

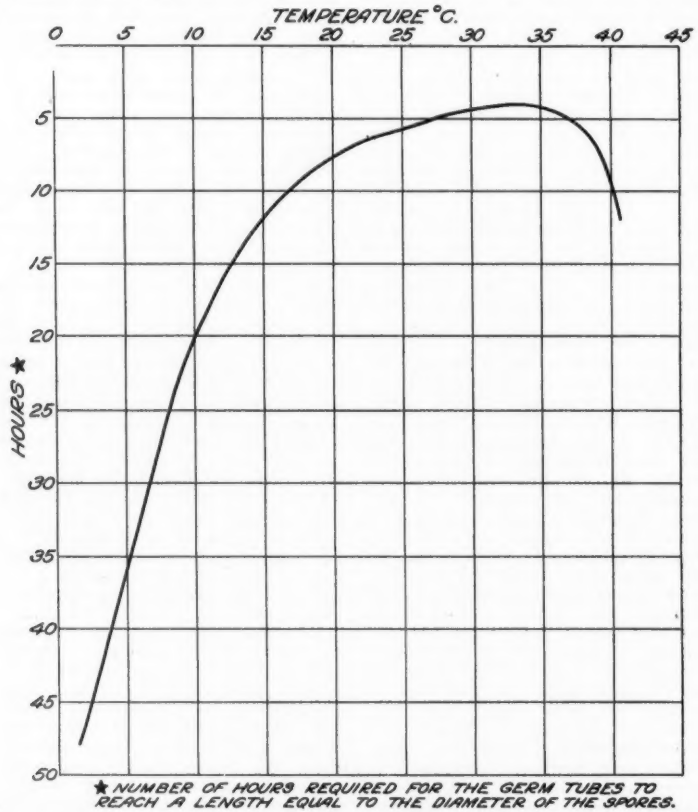


FIG. 1.—Curve showing the time in hours required for the spores to produce germ tubes equal in length to the diameter of the spores when incubated in sweet-potato decoction at different temperatures

No sharp optimum was noticeable for the first four days, but it became more pronounced thereafter. The optimum temperature tried was 26.8° C., although growth was only slightly less rapid at 22.5°, the next lower temperature. Above 26.8° the growth rate dropped abruptly. The optimum for mycelial growth lies somewhere between 25° and 27°, which is several degrees lower than that for spore germination. The optimum remained the

days at 38°, but a fair growth was made at 36°. Weimer and Harter (*18*) found that the more rapid-growing species of Rhizopus reached the highest thermal point of growth in the first 24 hours. Edson and Shapovalov (*6*) also found this to be the case with the fungi which they studied. However, as already pointed out, the brownrot fungus did not make a measurable amount of growth at the maximum temperature for several days. Never-

theless, this fungus grows very slowly at high temperatures and no doubt had made some growth several hours before a measurable disk was formed. This was also true as the minimum temperature was approached. No growth was evident at 2°, the lowest temperature tried, until after the eighth day. However, 5 and 13 mm. of growth were made at this temperature in 10 and 15 days, respectively. The minimum for growth is somewhere below 2°. Here again it is quite evident that the fungus can make considerable growth at the temperature existing in a refrigerator car in the week or two necessary for the shipment of the cauliflower from the Pacific coast to eastern markets.

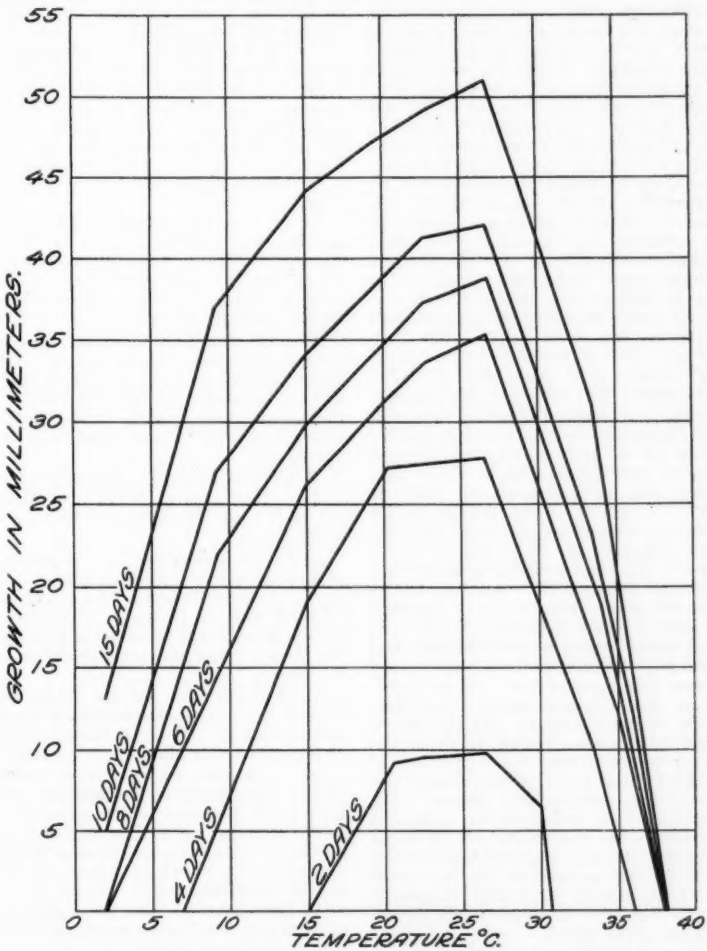


FIG. 2.—Graphs showing the diameter of the mycelial disks of *Alternaria brassicae* growing in Petri dishes on Irish potato agar at various temperatures after 2, 4, 6, 8, 10, and 15 days

The optimum temperature for fruiting as determined in these experiments was between 26° and 28°, while the maximum and minimum were practically identical with these temperatures for growth.

THE EFFECT OF TEMPERATURE AND HUMIDITY ON INFECTION

Young detached cauliflower leaves were employed to determine the influence of temperature upon infection. Because of continued catabolic activities and the retardation of anabolism, the leaves, after being removed from the plant, no doubt changed physiologically rather rapidly, especially at the higher temperatures. But, uninoculated control leaves at all but the highest temperatures remained turgid and retained their normal green color for several days longer than was necessary to obtain the desired results. Regardless of the change in the detached leaves, they more nearly approximated the normal plant than would any artificial culture medium which might have been used. The leaves were placed on moist cotton in small moist chambers in incubators at temperatures of 7°, 10°, 14°, 17°, 20°, 22°, 23.5°, 25.6°, 30°, 31°, 32.5°, 34°, 35.5°, 36.8°, and 38.2° C. The experiment was repeated with similar results. Abundant infection was evident after two days at all temperatures from 10° to 35.5° inclusive. Distinct lesions were present at 7° three days later, but no infection took place at 36.8° or above. The maximum for infection was about 36° since infection was obtained at 35.5° but none at 36.8°. The optimum for infection lay between 28° and 31°. Although there was not much difference in the number of lesions, they differed somewhat in size, being largest at the optimum and gradually becoming smaller above and below this temperature. At the optimum temperature aerial mycelium grew over the surface and the leaves gradually became yellow, while at the other temperatures the formation of aerial hyphae and the yellowing of the leaves was considerably slower. The optimum for infection of detached leaves under the conditions of these experiments was slightly higher than that for the growth of the mycelium on Irish potato agar and somewhat lower than that obtained for the germination of the spores.

Several experiments were conducted to determine the effect of temperature on infection and on the development of brownrot on the curd of cauliflower. The cauliflowers used were obtained from the city market at Washington, D. C., or were grown at the Arlington Experiment Farm at Rosslyn, Va. The curds were rinsed in tap water, placed in moist chambers on moist filter paper, sprayed with a heavy suspension of spores of *Alternaria brassicae* in distilled water, and then placed in a series of incubators whose temperatures ranged from 1.8° to 37.2° C. The incubators used were the same ones used in the spore germination and mycelial growth studies. Following are details of the effect of temperature on the infection of cauliflower curds by *Alternaria brassicae* and the development of brownrot:

At 1.8° infection became apparent in 5 to 7 days. After 14 days the lesions were chestnut brown, about 1 mm. in diameter and 0.5 mm. deep. At the end of 35 days the lesions had changed to an olivaceous color, due to the formation of spores. They were about 1 mm. in diameter by 1 to 2 mm. in depth.

At 7° numerous minute light brown lesions were apparent in 4 to 5 days. These were about 1 mm. in diameter by 1 to 2 mm. in depth in 19 days. In 35 days the lesions were 1 cm. in diameter by 1 to 1.5 cm. in depth.

At 9.5° abundant infection became evident in 3 days; these lesions were about 0.5 mm. in diameter and slightly more developed in 10 days. In 35 days the lesions were 2 to 3 cm. in depth and about half of the curd was involved.

At 10.5° infection became evident in 3 days and typical brownrot lesions 0.5 to 1.5 cm. in diameter developed in 20 days.

At 11° abundant but very small and superficial lesions became evident in 3 days. Tissues decayed to a depth of 0.5 to 1 mm. in 10 days. Practically the entire curd was involved in 35 days, the tissues being affected to a depth of 2 to 3 cm.

At 15.5° infection became evident in 3 days; the lesions were still quite small and superficial in 7 days, three-fourths of the curd being affected in 14 days.

At 18° lesions 0.5 mm. in diameter were evident in 48 hours, lesions 1 to 1.5 cm. in diameter were evident in 7 days, and the curd three-fourths involved in 14 days, the tissues being decayed to a depth of 1 cm. at the end of this time.

At 19.5° infection was just apparent in 24 hours; the lesions reaching 1 to 3 mm. in diameter in 3 days.

At 22.5° lesions were abundant but very small in 24 hours, about one-half of the curd being involved in 4 days. Nearly the entire curd was affected, the decay reaching a depth of 5 to 8 mm. in 7 days.

At 26.8° lesions were abundant in 24 hours and curd two-thirds involved in 4 days. Nearly the entire curd was affected, decay being 1 cm. deep in 7 days.

At 28.5° lesions were abundant in 24 hours. These were 1 mm. in diameter in 3 days, at which time bacterial rot was also present. The curd was nearly completely decayed by bacteria in 7 days.

At 30.7° lesions were abundant in 24 hours. These were about 1 mm. in diameter but were being obscured by bacteria in 2 days.

At 32° a few brownrot lesions, poorly developed, were evident in 3 days. Bacterial rot was present.

At 34.7° no brownrot developed in 3 days. The curd was decayed by bacteria.

At 37.2° no brownrot developed in 3 days. The curd was decayed by bacteria and Rhizopus.

Infection took place at all of the temperatures tried except 34.7° and 37.2° C. The disease developed more slowly at the lower than at the higher temperatures. The optimum temperature for infection lies somewhere between 25° and 30°. However, the optimum development of the disease occurred at about 25° since at the higher temperatures the heads were soon infected and decayed by bacteria. The number of lesions formed at any of the temperatures varied little except perhaps at 32°, where they were fewer. The infected areas in all cases were light brown in the early stages, gradually becoming darker, until they were olivaceous to nearly chestnut brown in color. The time necessary for the development of the aerial mycelium and spores which gave the olivaceous to brown color to the lesions varied with the temperature from about two days at the optimum to about 30 days at 1.8°. At 1.8° infection did not become apparent for from 5 to 7 days, at which time the lesions appeared as very minute lightbrown discolorations just visible to the unaided eye. Little progress had been made by the disease at the end of 19 days, as was indicated by a slight darkening in the color of the infected tissue. After 35 days the lesions had begun to take on the characteristic olivaceous color due to the presence of dark-colored aerial hyphae and spores. Nevertheless, at this time the decay had not penetrated over 1 to 2 mm. deep.

Infection was apparent in from 4 to 5 days at 7° and in 19 days the decay had extended into the tissue 1 to 2 mm. However, in 35 days the lesions were about 1 cm. in diameter and 1 to 1.5 cm. deep. At 9.5° to 15.5°, 18°, and 19.5° the maximum number of infections became evident in 3, 2, and 1 days, respectively. Although little actual decay was caused by this disease in two weeks below 10°, no doubt some decrease in the commercial value of the infected cauliflowers resulted from the browning of the curd.

The results of the experiments discussed above show quite definitely to what extent temperature may affect the development of brownrot on cauliflower under conditions of high humidity. An experiment was conducted in which the cauliflowers were sprayed with a heavy suspension of spores in water with an atomizer and then placed in wooden crates and held in large refrigerator rooms in a cold-storage house. The curds were fairly well enveloped by leaves. The temperatures of the rooms were 1°, 2.5°, 2.5°, and 4.5°, with a variation of perhaps 1° C. and a relative humidity of 81, 74, 91, and 87 per cent, respectively. Infection was visible in 10 days at all of the temperatures. But only a few lesions developed and these were still small at the end of a month. Under these conditions comparatively little damage resulted from this disease. This experiment was repeated later, the method being varied only in that the cauliflowers were placed in moist chambers with the expectation that the humidity of the air about the heads would thereby be raised. In this case the number of infections was greatly increased and the damage was proportionally greater. However, the fungus had not penetrated the tissues more than 0.5 cm. at any of the temperatures, there being little apparent difference in the stage of decay on the heads held at the different temperatures after five weeks.

EFFECT OF THE FUNGUS ON THE HOST

The blossoms of the cauliflower attacked by *A. brassicae* at first turn brown and take on a water-soaked appearance. This latter condition is especially noticeable in the larger branches of the curd. Very soon the lesions become olivaceous in color because of the presence of dark-colored aerial mycelium and spores. The mycelium is at first intercellular but later penetrates the cell walls and the diseased tissue is permeated with hyphae. The affected tissue is somewhat softened, although not so much so as in the bacterial rot. The decayed tissues lose water rather rapidly, so that the older lesions often have a dry and shriveled appearance. The fungus enters the leaves through the stomata and kills the tissues. This was demonstrated by placing drops of a suspension of spores upon a leaf in a moist

chamber. After 48 hours the inoculated spots were removed, fixed, dehydrated, infiltrated with paraffin, and sectioned. No evidence of direct penetration of the cell wall was seen. The cells showed a change in their reaction to stains very soon after the germ tubes had entered the stomata of the leaf. The cells soon died and the tissues affected turned dark. The extent to which the fungus spreads in the leaf varies greatly. It may form only a small dark spot about a millimeter in diameter, or it may spread throughout and kill a large area of the leaf.

PHYSIOLOGY

Chemical analyses (*5*) have shown that cauliflower contains the following carbohydrates: Cellulose, glucose, fructose, pentosans, and methyl pentosans. Dochnowski and Tollens (*5*) could not detect the presence of cane sugar but noted the existence of some hydrolyzable carbohydrates. On the other hand, Busolt (*2*) was unable to demonstrate the presence of glucose in cauliflower tissue but found mannit instead. Experiments were conducted to learn something of the carbohydrates which may serve as a source of carbon for *A. brassicae*. The carbohydrates tested were 5 and 10 per cent glucose, 1 per cent Irish potato starch, 5 per cent cane sugar, 5 per cent gum arabic and cellulose (dissolved and reprecipitated according to the method described by Scales (*15*)). Twelve 100 cc. Erlenmeyer flasks each containing 30 cc. of a modified Czapek's solution with the above carbohydrates substituted as a source of carbon were prepared and inoculated with a loop of a suspension of spores in sterile distilled water. The Czapek's solution was modified by the substitution of ammonium nitrate for sodium nitrate, as was found advantageous for the growth of Rhizopus by Weimer and Harter (*17*). One set of flasks had no carbohydrate present in order to determine if the fungus would grow on this solution without a carbohydrate being added. Ten of the 12 flasks of each set were inoculated and the two remaining held as controls. The flasks were held at about 30° in the dark for two weeks, after which observations were made on the amount and character of mycelial growth formed. There was not a sufficient amount of growth made in any of the flasks for dry-weight determinations. The presence of substances which did not go completely into solution, cellulose, starch, and gum arabic in particular made it impracticable to separate the mycelium from the medium by filtration; hence attempts to obtain the dry weight of the mycelium formed in each case for purposes of comparison were abandoned. Careful observations were made, however, which showed that practically no growth took place where carbon was not supplied. Glucose and cane sugar proved to be the best sources of carbon tried, followed closely by starch. Judging from the growth made, gum arabic can also serve as a source of carbon, but not nearly so effectively as the three substances mentioned above.

No more growth was made on the cellulose medium than in the controls where no carbon was present, which indicates that cellulose in this form can not be utilized by the fungus. In order to learn whether this fungus can hydrolize the starch and cane sugar, samples of the control solutions as well as those on which the fungus had grown were tested with Fehling's solution for the presence of reducing sugars. These tests were only comparative. Equal amounts of the culture media and of Fehling's solution (20 cc.) were used and the solutions were boiled approximately the same length of time in each case. The control cane-sugar solution showed only a trace of reduction, while some of the same solution on which the fungus had grown gave a heavy precipitate with Fehling's solution, there being more than enough reducing sugar present to reduce all of the copper. This indicates that the fungus produces a very active invertase. Similar tests with the solutions containing starch showed that there was no reducing sugar present in the control and only a very little in the inoculated solutions. Seemingly, the amylase produced, if any, was not sufficient to hydrolyze the starch much in excess of the needs of the fungus.

Since this fungus produces a rot of the cauliflower curd it is of interest to know whether an enzym capable of dissolving the middle lamellae is produced under artificial culture conditions. In order to determine this point, about 25 cc. of each of the above-mentioned solutions were placed in small flasks and small pieces of cauliflower curd and leaf and sweet potato were added. The sweet potato added was in the form of circular disks about 1 cm. in diameter by 0.5 mm. thick, cut from the fleshy root. The flasks were held at 45°. Five cc. of toluol were added to each flask as an antiseptic. Observations were made from time to time but no signs of maceration were evident in any case in 72 hours.

The above experiments indicate that a strong invertase; a weak amylase, if any; no cytase; and no pectinase were produced. To obtain further confirmation regarding the production of these enzyms by *A. brassicae*, fifty 200-cc. flasks, each containing 50 cc. of sweet-potato decoction, and a like number containing cabbage decoction, were prepared and inoculated. Several uninoculated flasks were held as controls. The Czapek's solution used in the earlier experiments did not appear to contain all the substances necessary for the best development of the fungus, hence these vegetable decoctions were used and proved to be very satisfactory; 500 gm. of the vegetables per liter were used in preparing the decoctions. Part of the inoculated flasks were held at 31° in the dark and the remainder were placed at room temperature (25°) and in diffused light. After 12 days a thick mycelial felt was present on all of the inoculated flasks. The amount of growth on these media was very much better than that made on Czapek's solution. The mycelial felt was very dark olive to almost black in color. Spores were present in great abundance. It might be added here that the fungus seems to sporulate even under the most adverse conditions, spores having been formed in considerable quantities even in Czapek's solution without carbon. Furthermore, it was found in the temperature experiments previously recorded that spores were formed at temperatures so high that they were extremely injurious to vegetative growth. In fact, sporulation seems to take place under any condition which will permit growth.

The felts were removed from the sweet potato and cabbage decoctions, both those in the light and in the dark. Each set was kept separate throughout. These felts were washed in running tap water for a few minutes to remove all traces of sugar and were then treated with acetone and ether in the manner described by Harter and Weimer (*10*). The hyphae were then weighed out in 0.5 gm. samples, each being placed in a small Erlenmeyer flask. Twenty-five cc. of distilled water were added to two flasks of each set. To these flasks were added disks, 1 cm. in diameter by 0.5 mm. thick, of sweet potato, carrot, and Irish potato and similar disks cut from the laminae of cauliflower leaves as well as small portions of the cauliflower curd. Twenty-five cc. of 0.75 per cent cane-sugar solution were added to each of two flasks containing hyphae grown on sweet-potato decoction in the light, to each of two flasks containing hyphae grown on sweet potato decoction in the dark, and to each of two flasks containing hyphae grown on cabbage decoction in the dark. Twenty-five cc. of a 0.75 per cent starch paste and 25 cc. of a cellulose suspension were added to each of two flasks containing hyphae from the same sources. Likewise, flasks containing the same amount of hyphae from the same sources with 25 cc. of distilled water added were held as controls. Samples of the cane-sugar solution, starch paste, and cellulose suspension were also held as controls; 5 cc. of toluol were added to all of the flasks as an antiseptic, and they were then held at 45°.

No maceration was evident in any of the flasks containing the hyphae and portion of vegetable in 96 hours, at which time the experiment was discontinued. Here, as in the former test, the presence of pectinase could not be demonstrated.

After 24 hours the flasks to which the cane sugar had been added were steamed and their contents tested for reducing sugars by the Clark method (*4*). At the end of 30 hours the flasks to which the starch paste and cellulose had been added were similarly treated and tested. The control flasks containing samples of the cane sugar, starch paste, and cellulose were also tested. Likewise, the control flasks containing hyphae and water were tested in the same way. The purpose of holding the hyphae in water was to determine the reducing sugar formed as a result of autolysis of the fungus itself. The average amount of reducing sugars present in the two flasks of each set minus that formed by autolysis and that in the original solutions are given in Table III. The data presented there show that a considerable quantity of both the cane sugar and the starch was hydrolyzed by the enzyms contained in the hyphae, denoting that invertase and amylase were produced by this fungus when growing on either sweet-potato or cabbage decoction. The number of tests made was not large enough to make the figures very accurate from a quantitative standpoint, but they are of value in that they show that considerable reducing sugar was produced in both the cane-sugar and starch solutions. Apparently, slightly more invertase was produced by the fungus when growing on cabbage than on sweet-potato decoction. However, this difference in the results obtained may be within the range of experimental error. On the other hand the reverse seemed to be true of amylase. Light appeared to have a detrimental effect on both enzyms.

It has been shown by Weimer and Harter (*19*) that acids were formed in the solutions on which species of Rhizopus grew in sufficient quantities to cause maceration of the sweet-potato disks. In order to be sure that the acidity or alkalinity of the media on which *A. brassicae* had grown was not such as to interfere with the enzym studies, the hydrogen-ion concentrations of the solutions were determined (electrometrically) and are given in Table IV. The figures in this table show that the cabbage decoction underwent slightly greater change in hydrogen-ion concentration than did the sweet-potato decoction. The change seemed to be slightly greater in the solutions on which the fungus had grown in the light. The data show that the hydrogen-ion concentration of none of the solutions was such as seriously to interfere with the pectinase tests made.

LIFE HISTORY

There seems to be little doubt that the causal organism (*A. brassicae*) lives over winter in the soil and in diseased parts of the host left on the ground. The results of experiments have shown that the spores will not only live but will germinate and grow at 2°. Although no experiments have been conducted to determine the minimum temperature which the spores will survive, there is every reason to believe that they will stand quite low temperatures. This is supported by the fact that this disease is prevalent in sections of the country where very low temperatures are experienced each winter. There is also the possibility that the fungus lives over winter on the seed as is shown by the following experiment:

Cauliflower seeds were dipped into a suspension of spores from a virulent culture of *A. brassicae*, then dried and stored in a drawer of a laboratory desk for eight months. Tests showed that some of the spores were then still capable of germinating. Some of the seeds dipped into the spore suspension were planted in a pan of sterilized soil in the greenhouse. A pan filled with some of the same soil was sown with seed from the same lot which had not been inoculated, as a control. A damping-off of the seedlings occurred in the pan sown with inoculated seed and *A. brassicae* was recovered from the diseased tissue a couple of centimeters above the surface of the ground. No damping-off occurred in the control pan. This experiment indicates that the disease may develop in the seed bed from the spores on the seed. In addition to attacking leaves, stems, and heads of cauliflower, the fungus also causes a spotting of the seed pods. It has been isolated from seeds taken from beneath the lesions on partly mature pods. Spores of the fungus have been seen on seeds taken from pods which had not yet begun to dehisce. This shows that the fungus can grow through the pods and infect the seeds. The fungus may be carried to the seed beds with such seeds and a damping-off of the seedlings produced. Spores are formed abundantly on the dead seedlings and serve as a source of inoculum for other plants. A few plants having small lesions on the lower leaves have been seen at transplanting time, showing that the disease may be carried from the seed bed to the field on the seedlings. This has not been seen very frequently and probably occurs only under conditions extremely favorable for the development of the fungus.

Inoculation experiments conducted in the greenhouse showed that the incubation period in the leaves was from 24 to 48 hours. The time necessary for infection to become evident varies with the temperature and humidity. Cauliflower plants with heads from 5 to 10 cm. in diameter growing in the field were inoculated on June 18, 1921. Infection took place and well developed lesions were present both on the leaves and curds in six days. At this time typical Alternaria spores were present in abundance both on the leafspots and the brownrot lesions. These spores served as a source of inoculum so that by the end of the season the disease was present on many of the neighboring uninoculated plants. The spores are easily separated from their sporophores, so that ample opportunity is thus afforded for the disease to become widespread in a comparatively short time, provided conditions are favorable for dissemination and infection.

MORPHOLOGY

The mycelium permeates the tissues of the head and leaf in all directions from the point of infection passing between and through the cells. The hyphae are hyaline at first, later becoming brownish to olivaceous. In culture mycelial development is suppressed, but on or in the curd of the cauliflower long profusely branched threads with numerous septations are developed. The hyphae vary in diameter on different substrates, ranging from 1.5 to 7.5 μ. They soon come to the surface of the host, finding an exit through stomata or wounds.

Under humid conditions the surface is soon covered with conidiophores bearing an abundance of spores. The conidiophores are short, olivaceous in color, usually concolorous with the spore, septate and branched. They are 5 to 7.5 μ wide by about 35 to 45 μ long. The spores are nearly linear to obclavate in shape and are borne on the conidiophores in chains, as many as 8 to 10 in a chain being quite common. The spores are produced abundantly both in culture and on the host. They are olivaceous to brown in color and together with the conidiophores impart the characteristic olivaceous color to the lesions on the host. They are smooth at first, becoming roughened with age, and have as many as 10 horizontal septations but few vertical ones. Spores from old cultures often have more septations and are thicker in proportion to their length and more obclavate than those in fresh cultures or those from fresh lesions. Spores with more than six horizontal septations are not numerous. Various spore types are shown in Figure 3.

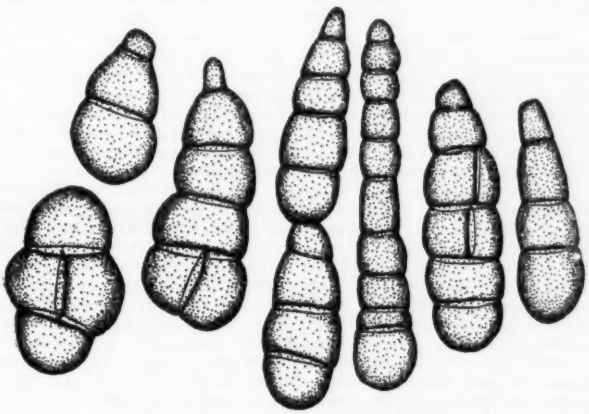


FIG. 3.—Various types of spores of *Alternaria brassicae*. ×1,000.

The spores taper somewhat toward the apex. The apical cell often is more or less rectangular in cross section, the outermost wall, however, being shorter than the wall contiguous with the penultimate cell. The basal cell is somewhat rounded. The maximum and minimum measurements for spores with different numbers of septations taken both from culture and from the host are as follows: 1, septate 6.5–7.5×11–13 μ; 2, septate 7.5–9.5×18.5–20.6 μ; 3, septate 9–11.2×26–41.25 μ; 4, septate 9–11.25×39.5–45.5 μ; 5, septate 9–13.9×37.5–45 μ; 6, septate 9–11.25×35.6–45 μ; 7, septate 13–16.8×56.25–65 μ; 8, septate 11.25–16.8×50–75 μ. The perfect stage of this fungus is unknown.

Elliott (*7*) discusses what he terms secondary development in Alternaria spores. This was also noticed by the writer. In the young cultures the spores are regular and smooth with few vertical cross walls. As the spores grow older the constrictions at the septa become deeper, the cells round off, the spores become darker in color, and the walls become roughened. This variation of the spore size and character has led to considerable confusion in the determination of *A. brassicae*.

CONTROL OF THE DISEASE

Alternaria leafspot of cauliflower and cabbage is of comparatively little importance during most seasons, so that ordinarily no remedial measures are necessary. However, spraying with Bordeaux mixture 4–4–50 with a sticker added is recommended in case of a severe outbreak (*16*).

The results of other workers seem to indicate that this disease may be held in check somewhat by treating the seed. Chupp (*3*) reports a 100 per cent killing of Alternaria on the seeds of cabbage by treating them with hot water at 56° for 10 minutes. However, the fungus could still be isolated from seeds treated with mercuric chloride. He likewise states that Walker found that treating cabbage seed with hot water at 50° for 30 minutes or 55° for 10 minutes will completely eliminate Alternaria.

As stated above, the disease on cauliflower plants growing in the field is not

sufficiently serious during most seasons to justify the application of control measures. However, a large number of spores may be produced on a comparatively few spots. These spores may infect the curd or may lie dormant until the heads are in transit. An attempt should be made to discard all heads with apparent lesions when the cauliflower is being crated, since such heads will have little if any market value after several days in transit. However, many inconspicuous lesions will be overlooked and also many more may originate during the shipping period. That the disease may be expected to develop during transit is shown by the fact that in the writer's experiments infection was evident at about 2° C. in from 5 to 7 days. The lesions, however, developed rather slowly, being about 1 mm. in diameter by 0.5 mm. in depth in 14 days under conditions of high humidity. At 7° the lesions were evident in from 4 to 5 days and had developed to 1 mm. in diameter by 1 to 2 mm. in depth in 19 days. These data indicated that it is possible for the disease to make considerable headway in the 10 days or thereabouts which must necessarily intervene between the time California cauliflower is placed in the car and the time it reaches its destination. The amount of loss caused depends upon the amount of infection and the number of spores on the heads at the time they are loaded into the car, as well as the temperature and humidity maintained in the car throughout the transportation period. In another experiment cauliflower inoculated and held at about 2° became infected, but the lesions were hardly visible to the unaided eye even after 20 days and would not have been recognized as such by the layman. In this experiment at temperatures varying from 6° to 7° C. the lesions were hardly recognizable at the end of 10 days and were still quite inconspicuous after 20 days.

From this and other experiments it seems that, although it is impracticable to hold cauliflower at a temperature which will inhibit infection during transportation, much can be accomplished by maintaining the temperature and relative humidity as low as possible. If the temperature is held at 5° C. or below throughout the transportation period of, say, 6 to 12 days, little injury will result from this disease. This is entirely practicable, as shown by tests with cantaloupes made by the Bureau of Agricultural Economics, United States Department of Agriculture (*12*). These showed that refrigerator cars loaded with cantaloupes at Brawley, Calif., did not reach a constant temperature level until about the end of the third day, after which a fairly uniform temperature was maintained. The temperatures in a couple of cars varied from about 1° to 6° C., depending on the part of the car in which the records were obtained. The cars were on the road 5 or 6 days and 12 days when en route to Chicago and to New York, respectively. Temperature records of other cars similarly loaded showed that after the cantaloupes became thoroughly cooled (about the third day) a temperature varying from about 4° to 10° C. could be maintained.

The cauliflower heads should be kept as cool and dry as possible after reaching their destination, and should reach the consumer without unnecessary delay. Precooling the cars and the cauliflower especially during warm weather before loading should be of material benefit in getting the temperature down and retarding the development of the disease during the early period of transportation.

The inspectors of the Bureau of Agricultural Economics reported the arrival of a car of cauliflower in Pittsburgh on November 21, 1921, which had been shipped from Orchard Park, N. J. About one-third of the curds showed brownrot in varying degrees of severity; some had only a few small spots, while others were almost entirely covered with them. The temperature of this car taken at the time the inspection was made was 8° at the bottom and 9° C. at the top. Although these figures are of little value in showing the temperature condition of the car during transit, the condition of the cauliflower indicates that the disease can develop in transit when hauled only a comparatively short distance if the conditions are favorable. No record of the time elapsing between the dates of loading and of unloading the car was obtained.

The data presented in this paper seem to justify the following recommendations for controlling the brownrot of cauliflower:

Prevent field inoculation and infection by keeping the disease under control on the growing plants. With this in view, practice seed treatment, sanitary seed-bed preparation, and crop rotation. If the disease becomes at all prevalent the plants should be sprayed with 4–4–50 Bordeaux mixture.

Pack and ship only cauliflower entirely free from the disease.

Ship in good refrigerator cars, which are kept at as low a temperature as possible (7° C. or below) throughout the transportation period by refilling

TABLE I.—*Amount of brownrot found by inspectors of the Bureau of Agricultural Economics on cauliflower in various shipments*

Date	Source	Market	Percentage of heads affected
Dec. 12, 1920	Los Angeles, Calif.	Chicago, Ill.	3–5
Mar. 23, 1920	do	Fort Worth, Tex.	50–100
Feb. 13, 1920	do	Chicago, Ill.	20–30
Feb. 7, 1920	do	do	100
Jan. 1, 1920	Compton, Calif.	do	0–20
Feb. 6, 1920	Bradentown, Fla.	do	80
Mar. 22, 1919	San Francisco, Calif.	New York, N. Y.	10

TABLE II.—*Detailed data on experiments conducted to prove the pathogenicity of A. brassicae isolated from different types of lesions on different hosts and from different sources*

Number of organism	Source of organism	Date of inoculation	Host	Part of plant used	Number of plants inoculated	Number of plants infected	Number of controls	Number of controls infected
		1921						
4746c	Cauliflower curd, Washington, D. C., city market.	Jan. 8	Cauliflower	Curd	2	2	1	0
4829	Pure line of 4746c	Mar. 12	do	do	2	2	1	0
4829	do	do	Cabbage	Head	2	2	1	0
4829	do	Mar. 14	Cauliflower	Seedlings	(a)	(b)	(c)	0
4829	do	Mar. 31	do	do	(d)	(b)	(a)	0
4829	do	do	do	Half-grown plants.	3	3	1	0
4829	do	do	Cabbage	do	3	3	2	0
4829	do	Apr. 4	Cauliflower	Curd	1	1	1	0
4828	Cauliflower leaf, California	do	do	do	1	1	1	0
4866a	Cauliflower curd, California	do	do	do	1	0	1	0
4829	Pure line of 4746c	June 2	do	Plants in field, curd.	7	7	7	0
4828	Cauliflower leaf, California	June 18	do	do	5	5	5	0
4829	Pure line of 4746c	do	do	do	10	10	5	0
4829	do	do	do	do	8	8	6	0
4828	Cauliflower leaf, California	do	Cabbage	Plants in field	5	5	6	0
4829	Pure line of 4746c	July 4	do	do	10	10	5	0
4828	Cauliflower leaf, California	Nov. 10	Cauliflower	Curd	1	1	1	0
4829	Pure line of 4746c	do	do	do	1	1	1	0
4973d	Cauliflower curd, Washington, D. C.	do	do	do	1	1	1	0
4866a	Cauliflower curd, California	do	do	do	1	0	1	0
5047	Washington, D. C., city market.	do	do	do	1	1	1	0
4997c	Cabbage leaf, Arlington, Va.	do	do	do	1	1	1	0
		1922						
5104	Cabbage from New York, in storage at Washing- D. C.	Feb. 22	do	Plant 1 foot high.	2	2	2	0
4829	Pure line of 4746c	do	do	do	2	2	2	0
4997c	Cabbage leaf, Arlington, Va.	do	do	do	2	2	2	0
4828	Cauliflower leaf, California	do	do	do	2	2	(e)	0
4973d	Cauliflower curd, Washington, D. C.	do	do	do	2	2	(e)	0
5093	Cabbage leaf	do	do	do	2	2	(e)	0
5091	Cauliflower curd, Florida	do	do	do	2	2	(e)	0
5095	Cauliflower leaf; not typical Alternaria spot.	do	do	do	2	0	(e)	0
5089	Cabbage leaf	do	do	do	2	0	(e)	0
4866a	Cauliflower curd, California	do	do	do	2	0	(e)	0

[a] 2 pans. [b] Large number. [c] 1 pan. [d] 4 pans. [e] Same control as above.

TABLE III.—*The quantity of reducing sugars in mg. per 10 cc. resulting from the hydrolysis of cane sugar, starch, and cellulose by 0.5 gm. of hyphae of A. brassicae in 25 cc. of solution*

Substances tested	Hyphae grown on sweet-potato decoction		Hyphae grown on cabbage decoction in the dark
	In the dark	In the light	
0.75 per cent cane-sugar solution	91.38	82.66	95.58
0.75 per cent starch paste	68.80	58.14	61.05
Cellulose	00.00	00.00	00.00

TABLE IV.—*The hydrogen-ion concentration of cabbage and sweet-potato decoctions on which A. brassicae had grown for 12 days and of the uninoculated controls*

Solutions	Treatment	P_H
Cabbage decoction	Uninoculated control, held in the dark	5.24
Do	Uninoculated control, held in the light	5.01
Do	Inoculated, held in the dark	7.27
Do	Inoculated, held in the light	7.54
Sweet-potato decoction	Uninoculated control, held in the dark	4.96
Do	Uninoculated control, held in the light	4.96
Do	Inoculated, held in the dark	6.46
Do	Inoculated, held in the light	6.58

the ice bunkers at frequent intervals. The addition of salt to the ice in the bunkers and the placing of ice in and over the load will help to keep the temperature down. Precooling the cars as well as the cauliflower before loading should prove beneficial, especially when shipments are made during warm weather.

SUMMARY

(1) The leafspot of cauliflower caused by *Alternaria brassicae* has been studied, the pathogenicity of the casual fungus has been established, and the symptoms produced on plants of different ages and under different conditions described.

(2) A decay of the curd of cauliflower, called "brownrot", is also produced by *A. brassicae*. This disease of the head is found only when the cauliflower is overmature or when the head has been cut from the stalk. The disease is most destructive on cauliflower heads in transit where they are decayed or discolored, so that their market value is greatly reduced.

(3) *A. brassicae* has an optimum temperature for spore germination of 33° to 35° C. The maximum temperature for spore germination lies somewhere between 40° and 46° and the minimum is below 1.5°.

(4) The optimum temperature for the growth of the mycelium in Petri dishes on Irish potato agar lies between 25° and 27°. The fungus made some growth at 36° but none at 38°. No growth was evident at 2°, the lowest temperature tried, until after the eighth day. The minimum for growth is somewhere below 2°, although growth is very slow at this temperature.

(5) The maximum temperature for infection of detached cauliflower leaves, artificially inoculated and held in moist chambers at various temperatures, was about 36°. The optimum for infection under the same conditions lay between 28° and 31°. Infection was evident at 7° in five days.

(6) The optimum temperature for infection of the heads inoculated and held at various constant temperatures in moist chambers was between 25° and 30°. No infection took place at 34.7° or above. Infection did not become apparent at 1.8° for from 5 to 7 days, at which time the lesions appeared as very minute light-brown discolorations just visible to the unaided eye. The disease had developed but little at the end of 19 days. Infection was apparent in from 4 to 5 days at 7°, and in 19 days the decay extended into the tissue 1 to 2 mm. Although little actual decay was caused by this disease in two weeks below 10°, some decrease in the commercial value of the infected cauliflower resulted from the browning of the curds. The amount of infection and the rate of development of the disease increase as the humidity of the surrounding air is raised.

(7) Enzym studies showed that *A. brassicae* produced invertase and amylase but not pectinase or cytase, at least in demonstrable amounts, when growing on sweet-potato and on cabbage decoctions. These decoctions were made less acid by the fungus.

(8) *A. brassicae* attacks the leaves, stems of seedlings, heads, seed pods, and seeds of cauliflower. It has been isolated from seeds taken from within infected immature pods, showing that it can grow through the pod into the seeds. The fungus can be carried to the seed bed on the seeds, where it can cause damping-off of the young seedlings. Spores are produced abundantly on seedlings killed in this manner and these may serve as a source of further infection. The fungus can no doubt also live from one season to another in the soil.

(9) Suggestions are made for controlling the disease.

LITERATURE CITED

(1) BERKELEY, M. J.
1836. FUNGI. 386 p. London. (Smith, J. E., English Flora, v. 5, pt. 2. Also forms, v. 2, pt. 2, of W. J. Hooker's British flora.)

(2) BUSOLT, E.
1913. BEITRÄGE ZUR KENNTNIS DER KOHLENHYDRATE DER GEMÜSEARTEN. MITTEILUNG II. BEITRÄGE ZUR KENNTNIS DER IM SAFT DER GRÜNEN SCHNITTBOHNEN ENTHALTENEN KOHLENHYDRATE. Jour. Landw. 61: 153–160.

(3) CHUPP, C.
1923. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr. Bur. Plant Indus., Plant Disease Surv. Bul. Suppl. 26, 163 p., illus. [Mimeographed.]

(4) CLARK, W. B.
1918. VOLUMETRIC DETERMINATION OF REDUCING SUGARS. A SIMPLIFICATION OF SCALES' METHOD FOR TITRATING THE REDUCED COPPER WITHOUT REMOVING IT FROM THE RESIDUAL COPPER SOLUTION. Jour. Amer. Chem. Soc. 40: 1759–1772, illus.

(5) DOCHNOWSKI, R., and TOLLENS, B.
1910. ÜBER DIE BESTANDTEILE DES BLUMENKOHLS. Jour. Landw. 58: 27–31.

(6) EDSON, H. A., and SHAPOVALOV, M.
1920. TEMPERATURE RELATIONS OF CERTAIN POTATO-ROT AND WILT-PRODUCING FUNGI. Jour. Agr. Research 18: 511–524, illus.

(7) ELLIOTT, J. A.
1917. TAXONOMIC CHARACTERS OF THE GENERA ALTERNARIA AND MACROSPORIUM. Amer. Jour Bot. 4: 439–476, illus.

(8) FAWCETT, H. S.
1909. CABBAGE DISEASES. Fla. Agr. Exp. Sta. Ann. Rpt. 1909: 59–60.

(9) HARTER, L. L., and JONES, L. R.
1918. CABBAGE DISEASES. U. S. Dept. Agr. Farmers' Bul. 925, 30 p., illus.

(10) ——— and WEIMER, J. L.
1921. STUDIES IN THE PHYSIOLOGY OF PARASITISM WITH SPECIAL REFERENCE TO THE SECRETION OF PECTINASE BY RHIZOPUS TRITICI. Jour. Agr. Research 21: 609–625.

(11) HIGGINS, B. B.
1917. NOTES ON SOME DISEASES OF COLLARDS. Ga. Agr. Exp. Sta. Ann. Rpt. (1916) 29: 21–27, illus.

(12) MCKAY, A. W., FISCHER, G. L., and NELSON, A. E.
1921. THE HANDLING AND TRANSPORTATION OF CANTALOUPES. U. S. Dept. Agr. Farmers' Bul. 1145, 23 p., illus.

(13) PUTTEMANS, A.
1912. NOUVELLES MALADIES DE PLANTES CULTIVÉES. Bul. Soc. Roy. Bot. Belg. (1911) 48: 235–247, illus.

(14) SACCARDO, P. A.
1886. SYLLOGE FUNGORUM. v. 4. Patavii.

(15) SCALES, F. M.
1915. A NEW METHOD OF PRECIPITATING CELLULOSE FOR CELLULOSE AGAR. Centbl. Bakt. (II) 44: 661–663.

(16) SHERBAKOFF, C. D.
1917. SOME IMPORTANT DISEASES OF TRUCK CROPS IN FLORIDA. Fla. Agr. Exp. Sta. Bul. 139, p. 192–277, illus.

(17) WEIMER, J. L., and HARTER, L. L.
1921. GLUCOSE AS A SOURCE OF CARBON FOR CERTAIN SWEET-POTATO STORAGE-ROT FUNGI. Jour. Agr. Research 21: 189–210.

18) ——— ———
1923. TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS. Jour. Agr. Research 24: 1–40, illus.

(19) ——— ———
1923. HYDROGEN-ION CHANGES INDUCED BY SPECIES OF RHIZOPUS AND BY BOTRYTIS CINEREA. Jour. Agr. Research 25: 155–164.

THE DUSTFALL OF FEBRUARY 13, 1923 [1]

By Alexander N. Winchell, *Professor of Mineralogy and Petrology, University of Wisconsin*, and Eric R. Miller, *Meteorologist, United States Weather Bureau*

INTRODUCTION

Dustfalls seem relatively rare. During the past six years the catch of every rain and snow at Madison, Wis., has been filtered, but samples from only four dustfalls have been obtained. Except in those four well-defined cases, the solid precipitates were very small in amount and consisted mostly of soot and other products of combustion. Reports on the dustfall of March 9, 1918, and of a second on March 19, 1920, have already been published by the writers (*11*, *12*).[2] The third dustfall, on February 13, 1923, is discussed in the present paper. The unusually complete samples afforded by the fourth dustfall, on March 29, 1924, are now being prepared for analysis.

DESCRIPTION OF THE DUSTFALL

The dustfall of February 13, 1923, differed from the other three in that the region of deflation extended nearer to Madison, Wis., on this occasion. The dust fell at Madison in a snowfall of 0.6 inch which fell as the center of Low No. IV passed over. Figure 1 shows the track of this storm. At Madison the accompanying precipitation lasted from 1.30 to 4.30 p. m. The direction and velocity of the wind during this time were as follows:

Time (p. m.)	*Direction*	*Miles per hour*
1 to 2	Northeast	5
2 to 3	West	7
3 to 4	Northwest	10
4 to 5	West	27

Velocities averaging 25 miles per hour continued for the following 24 hours.

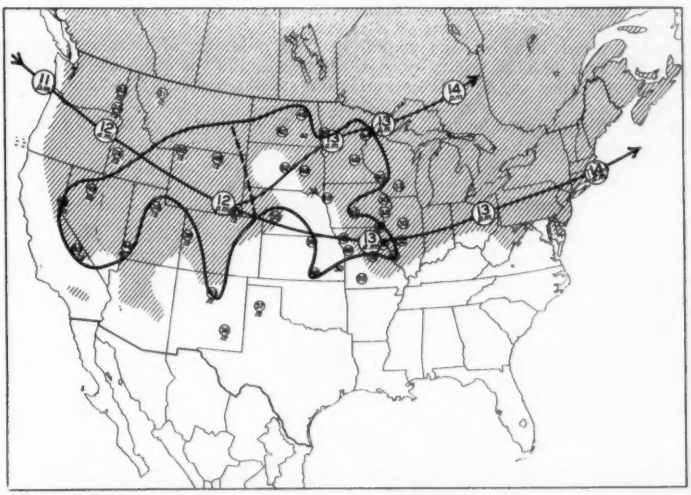


Fig. 1.—Shaded area shows extent of snow cover at 7 p. m. February 12, 1923. Forked line with arrowheads shows paths of centers of dust-bringing whirlwind. Numbers within small circles show maximum wind velocities in miles per hour. The day of occurrence was February 13 except at places marked 12 below the circle, where it occurred on the 12th. The closed line includes all places where the maximum velocity exceeded 40 miles per hour

[1] Received for publication May 6, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 450.

The rapid rise of velocity after the passing of the storm center was produced by the steep barometric gradient between Low IV and High VIII which followed closely. These strong following winds characterized this storm all the way across the western half of the continent. The closed loop in Figure 1 shows the area within which the wind attained velocities exceeding 40 miles per hour at all stations. These velocities occurred west of the dividing line on February 12, and east of that line on February 13. The winds apparently blew the dust up into the air, where it was carried forward into and around the storm center, and there precipated with rain and snow. Air trajectories of this type have been traced by Shaw and Lempfert (*9, figs. 84 K and 86 B*).

Snow covered most of the region within the area of high winds, except the Missouri valley (fig 1). Trees and other tall vegetation exposed to the wind throughout the area doubtless contributed to the organic matter shown by the analyses. The dust-bearing snow was so badly drifted that no attempt was made to ascertain the weight of the dust per unit area. The samples used in the analyses were collected in the country west of Madison by W. S. Fusch and Dr. J. G. Dickson. Director Edwin B. Frost, of the Yerkes Observatory, also forwarded large samples obtained by sweeping the terrazzo floor of the observatory, into which the wind had blown dust through crevices.

PHYSICAL COMPOSITION

Prof. H. W. Stewart, of the Soils Department of the University of Wisconsin, made a mechanical analysis of the dust. His results are given in Table I.

The breaking of a tube prevented completion of the duplicate on the clay and silt separates. The "fine gravel," "coarse sand," and "medium sand" are practically all plant tissues and not mineral matter. There is some organic

TABLE I.—*Size of constituents of dustfalls in a 10-gm. sample of air-dried material*

Separates	Size	Per cent in duplicate parts		Average
		A	B	
Fine gravel	2.0–1.0	0.15	0.10	0.12
Coarse sand	1.0–0.5	0.40	0.41	0.41
Medium sand	0.5–0.25	1.10	0.98	1.04
Fine sand	0.25–0.1	4.37	3.74	4.06
Very fine sand	0.1–0.05	33.17	33.36	33.26
Coarse silt	0.05–0.025	14.15		14.15
Medium silt	0.025–0.01	33.76		33.76
Fine silt	0.01–0.0005	3.79		3.79
Clay	Less than 0.005	10.60		10.60
Total		101.49		101.19

TABLE II.—*Size of constituents of dustfalls compared with that of loess*

Size	Percentages				
	A	B	C	D	E
<0.005	10.60	25.46	11.15	24.5	28.9
0.005–0.010	3.79	12.04	22.01		
0.010–0.025	33.76	44.60	56.17	66.5	63.9
0.025–0.050	14.15	11.47	5.99		
0.05–0.10	33.26	4.96	1.22	5.8	4.6
0.10–0.25	4.06	.82	1.04	1.0	1.3
0.25–0.50	1.04	.04	0.58	0.4	0.4
0.50–1.0	0.41	.02	0.29	1.2	0.6
1.0–2.0	0.12	.00	1.08	0.6	0.2
Total	101.19	99.41	99.53	100.0	99.9

A.—Dust from snowfall at Madison, Wis., Feb. 13, 1923.
B.—Average of three samples of dustfall at Madison, Wis., Mar. 19, 1920 (*12, p. 349*).
C.—Dustfall at Madison, Wis., Mar. 9, 1918 (*11, p. 602*).
D.—Loess, 3 feet below surface, Muscatine Co., Iowa (*3, p. 1848*).
E.—Loess, 3 feet below surface, Ringgold Co., Iowa (*2, p. 1918*).

matter in the other parts, but the percentage is very small.

It may be useful to compare these results with similar analyses of other dustfalls and of loess from the upper Mississippi valley, as shown in Table II and in Figure 2. Many other mechanical analyses of loess from the no doubt that part of it is actually present in the ferrous state. The only analysis of another American dustfall with which this can be compared is that of an earlier fall at Madison. As only a few satisfactory analyses of foreign dustfalls are available, all the good ones are included in Table IV.

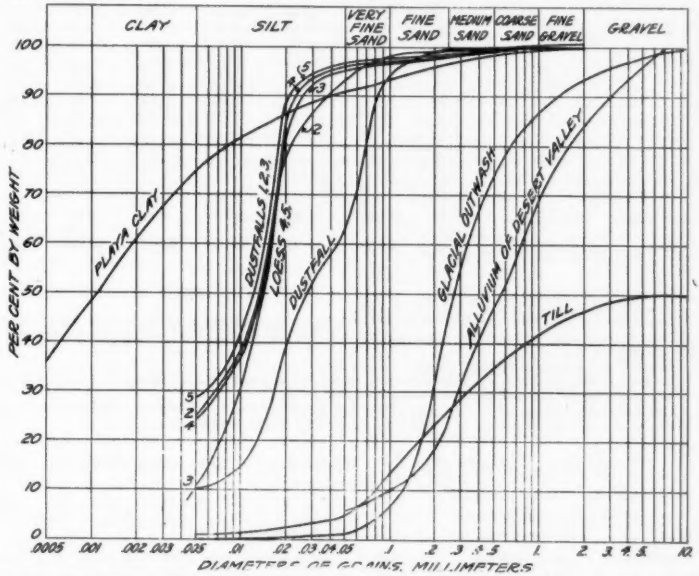


FIG. 2.—Mechanical composition of dustfalls and of natural soils. The ordinate shows the per cent by weight of all sizes smaller than the size indicated by the abscissa. Curves 1 to 5 refer to Table II. Other curves from Meinzer, Water Supply Paper 489

Mississippi Valley could be given, but they simply show that it varies considerably in the abundance of the various sizes. Increase of examples would not modify the main conclusion, which is that there is no essential difference in this respect between the material of dustfalls and that composing loess.

CHEMICAL COMPOSITION

Dr. E. J. Graul, of the Soils Department of the University of Wisconsin, made a determination of the alkalies in the dust. The main analyses were carried out in duplicate by Martin Tosterud, of the Chemistry Department of the University.[3] The results are shown in Table III. It was impossible to determine the state of oxidation of the iron in the dust because of organic matter. The small amount present was determined after complete oxidation, and is so reported, but there is

TABLE III.—*Chemical composition of the Madison dustfall of February 13, 1923 (dried at 105° C.)*

Constituents	Percentages		
SiO_2	71.75	71.70	71.73
Al_2O_3	7.84	7.85	7.85
Fe_2O_3	3.85	4.01	3.93
FeO			
MgO	1.01	.99	1.00
CaO	1.83	1.82	1.83
Na_2O	1.12	1.22	1.17
K_2O	2.15	2.06	2.11
H_2O (above 105° C.)	5.00	4.89	4.95
TiO_2	.48	.49	.49
P_2O_5	.17	.17	.17
MnO	.24	.17	.21
N	.37	.37	.37
Ignition [a]	3.57	3.65	3.61
	99.38	99.39	99.42
H_2O (below 105° C.)	1.65	1.66	1.66
H_2O (below 110° C.)	2.46	2.45	2.46

[a] Includes organic matter and CO_2, but not H_2O nor N.

[3] In order to further this work a grant of funds for chemical analyses was obtained from the research fund of the University of Wisconsin.

TABLE IV.—*Chemical composition of Madison dustfalls compared with that of foreign dustfalls*

Constituents	Percentages						
	A[a]	B	C	D	E	F	G
SiO_2	71.73	67.20	53.68	45.94	45.40	41.43	36.32
Al_2O_3	7.85	13.71	18.44	18.35	19.97	10.38	16.35
Fe_2O_3	3.93	2.17	6.54	6.57	7.03	9.19	6.08
FeO							
MgO	1.00	1.76	1.52	1.86	3.13	.92	2.21
CaO	1.83	1.74	.95	8.64	6.50	14.10	6.24
Na_2O	1.17	2.11	1.67	1.16	2.61	1.66	2.59
K_2O	2.11	2.30	2.58	2.30	2.07	1.58	2.72
H_2O	4.95	3.22	----	----	----	----	----
CO_2	----	----	----	6.10	3.46	8.45	3.68
TiO_2	.49	.52	----	----	----	----	----
P_2O_5	.17	.15	----	----	.20	----	----
MnO	.21	.38	----	----	----	----	----
N	.37	.39	----	.16	----	----	.16
Ignition	3.61	5.62	14.60	6.73	8.19	5.14	13.44
Total	99.42	101.28	99.98	100.00	99.73	99.00	100.00

[a] A.—Dustfall at Madison, Feb. 13, 1923.
B.—Dustfall at Madison, Mar. 19, 1920 (*12, p. 349*).
C.—Dustfall at Otakaia, New Zealand, Nov. 14, 1902, which came about 1,500 miles from Australia (*6*).
D.—Dust in "red rain" fall at Lamberhurst, England, Feb. 22, 1903; 2.19 per cent of organic carbon included in total (*10, p. 54*).
E.—Dustfall at Naples, Italy, Mar. 10, 1901. (*7, p. 157.*)
F.—Dustfall at Naples, Italy, Feb. 25, 1879, analysis by Scacchi, quoted by Palmeri (*7, p. 161*); 4.16 per cent of organic material and 1.39 per cent "loss" included in total.
G.—Dustfall at Taormina, Sicily, Mar. 19, 1901. The fall amounted to 5½ tons per square mile (*10, p. 222*); 9.89 per cent of organic carbon and 0.32 per cent of CoO included in total.

TABLE V.—*Chemical composition of Mississippi Valley loess compared with that of Madison dustfalls*

Constituents	Percentages							
	A[a]	B	C	D	E	F	G	H
SiO_2	79.77	74.46	72.68	71.73	70.86	70.11	67.20	64.61
Al_2O_3	9.95	12.26	12.03	7.85	8.91	14.25	13.71	10.64
Fe_2O_3	3.39	3.25	3.53	3.93	2.97	4.02	2.17	2.61
FeO		.12	.96		.10			.51
MgO	.26	1.12	1.11	1.00	3.12	1.32	1.76	3.69
CaO	.67	1.69	1.59	1.83	4.13	1.53	1.74	5.41
Na_2O	1.08	1.43	1.68	1.17	1.69	1.09	2.11	1.35
K_2O	2.05	1.83	2.13	2.11	1.18	2.03	2.30	2.06
H_2O	2.55	2.70	2.50	4.95	1.10	2.48	3.22	2.05
CO_2	----	.49	.39	----	4.70	----	----	6.31
TiO_2	.70	.14	.72	.49	.59	----	.52	.40
P_2O_5	----	.09	.23	.17	.40	----	.15	.06
MnO	----	.02	.06	.21	.28	----	.38	.05
Total	100.42	99.83	100.22	99.42	99.98	100.33	101.28	100.06

[a] A.—Loess, Terre Haute, Ind. (*8, p. 562*).
B.—Loess, Kansas City, Mo. (*1, p. 282*). 0.12 organic carbon, 0.06 SO_3 and 0.05 Cl included in total.
C.—Loess, Dubuque, Iowa (*1, p. 282*). 0.51 SO_3, 0.09 organic carbon, and 0.01 Cl included in total.
D.—Dustfall at Madison, Wis., Feb. 13, 1923. 3.61 organic carbon and CO_2 and 0.37 N included in total.
E.—Loess, Mt. Vernon, Iowa (*4*).
F.—Loess, Kansas City, Mo. (*5, p. 94*). 3.50 ignition loss included in total.
G.—Dustfall at Madison, Wis., March 19, 1920 (*12, p. 349*). 5.62 ignition loss and 0.39 N included in total.
H.—Loess, Galena, Ill. (*1, p. 282*). 0.13 organic carbon, 0.11 SO_3 and 0.07 Cl included in total.

The dustfall at Madison on February 13, 1923, is the most siliceous on record, although some dustfalls in Europe derived from the Sahara desert are said to reach a maximum of 73.45 per cent of silica.

European dustfalls are often very red, producing the so-called "red rain," or "rain of blood." This is due to complete oxidation of the iron, and is in marked contrast to the incomplete oxidation and gray color of the

Madison dustfalls. The latter also differ from the European dustfalls in the relative scarcity of calcium carbonate. The only foreign dustfall which resembles the American material in this respect is that of New Zealand (Table IV, C.)

In chemical composition the Madison dustfalls resemble the loess of the Mississippi Valley more closely than the dust from the African desert. This is shown in Table V.

The loess from Mount Vernon, Iowa (Table V, E), and from Galena, Ill. (*H*), contains considerably more lime carbonate than the Madison dustfalls (D, G). But in other respects the loess and the dust are strikingly similar. The dust of the 1923 fall (D) is more like the loess in chemical composition than is the dust of the 1920 fall (G).

MINERAL COMPOSITION

It is impossible to determine microscopically the mineral components which form the finest "clay" particles of the dust, but those particles of the size of "very fine sand" (0.05 to 0.10 mm.) can be recognized with little difficulty. In the dustfall of 1923 such particles form one-third of the whole dust, by weight, and therefore give an approximate idea of the mineral composition of the whole, since the particles of different sizes are not radically different in composition. A microscopic examination of the "very fine sand" disclosed the presence of very abundant quartz grains, a few feldspar grains, rather common limonite aggregates and grains thoroughly stained with limonite (which are hardly distinguishable from brown hydrocarbon fragments), and very minor amounts of hornblende, chlorite, carbonate, garnet, zircon, epidote, zoisite, apatite, tourmaline, hematite and magnetite. The feldspar is chiefly microcline with some albite. Most of the material is clear and readily identified except for that which is stained by limonite.

It is possible to calculate the approximate mineral composition from the gross chemical composition by making certain assumptions similar to those made in calculating the mineral composition of igneous rocks. It is assumed that all the soda is in albite feldspar, all the potassium is in orthoclase (or microcline) feldspar, all the phosphoric acid is in apatite, all the titanic acid is in ilmenite, all the magnesia is in chlorite (in case of insufficient alumina the magnesia may be assigned to enstatite), all the lime remaining after forming apatite and calcite is in anorthite feldspar, all the alumina remaining after forming feldspars and chlorite is in kaolinite, and all the silica remaining after forming all these silicates is in quartz. These assumptions are not all true, but they represent approximations and possible (even if not actual) combinations of the oxides into minerals. They furnish a useful means of comparing analyses, especially when the underlying assumptions are based so far as possible on determination of mineral composition by microscopic study. The results of such computations so far as they relate to dustfalls, are given in Table VI.

Table VI shows even more plainly than Table IV that the Madison dustfalls are exceptionally rich in quartz; they contain about as much feldspar as the New Zealand and one of the Naples dustfalls. The tenor of calcite and hematite is much lower than in the European dusts.

Table VII shows the degree of similarity between the mineral composition of the Madison dustfalls and that of loess of the Mississippi Valley.

It is evident that while the Madison dustfalls contain two to four times as much free silica (quartz) as foreign dustfalls they contain about the same tenor as found in loess of the upper Mississippi Valley. The 1920 dustfall contained more feldspar and less kaolin than the loess samples, but these differences do not recur in the 1923 dustfall. Some samples of loess contain abundant carbonate, but others contain little or none, like the dustfalls.

Dr. J. G. Dickson, of the Department of Plant Pathology of the University of Wisconsin, collected and examined five samples of dust-bearing snow, to determine the number of plant disease spores present. The samples were obtained in several locations 4 miles south and west of Madison, Wis., on the bench land. A layer of snow-bearing dust was scraped up without taking the bulk of snow either above or below. This was melted, and 10 cc. centrifuged. Spores were counted by means of a haemacytometer. The results are given in Table VIII.

TABLE VI.—*Calculated mineral composition of American and European dustfalls*

Constituents	Percentages						
	A[a]	B	C	D	E	F	G
Quartz	52.27	36.47	18.36	13.34	5.75	17.32	
Albite	9.91	17.89	14.21	9.82	22.14	14.04	21.98
Orthoclase	12.50	13.69	15.32	13.64	12.28	9.38	16.15
Anorthite	8.01	7.64	4.73	4.38	4.44	16.17	7.75
Kaolinite	.43	7.94	26.20	28.85	25.22		13.01
Chlorite	2.77	9.26	4.20	5.13	8.66		6.11
Enstatite						2.30	
Calcite				13.86	7.85	19.17	8.36
Apatite	.39	.35			.46		
Anhydrite					2.33		
Hematite	3.70		6.54	6.57	7.03	9.19	6.40
Ilmenite	.93	1.01					
Water, etc	8.51	7.03	10.42	4.41	4.41	11.43	20.89
Total	99.42	101.28	99.98	100.00	100.57	99.00	100.65

[a] A—Dustfall at Madison, Wis., Feb. 13, 1923.
B—Dustfall at Madison, Wis., Mar. 19, 1920.
C—Dustfall at Otakaia, New Zealand, Nov. 14, 1902.
D—Dust in "red rain" at Lamberhurst, England, Feb. 22, 1903.
E—Dustfall at Naples, Italy, Mar. 1, 1901.
F—Dustfall at Naples, Italy, Feb. 25, 1879.
G—Dustfall at Taormina, Sicily, Mar. 10, 1901.

TABLE VII.—*Calculated mineral composition of Madison dustfalls compared with that of the Mississippi Valley loess*

Constituents	Percentages							
	A[a]	B	C	D	E	F	G	H
Quartz	58.58	52.27	49.50	48.89	45.56	43.52	39.66	36.47
Albite	9.14	9.91	12.10	14.30	14.22	9.24	11.42	17.89
Orthoclase	12.16	12.50	10.86	6.98	12.62	12.03	12.20	13.89
Anorthite	3.34	8.01	4.74		2.19	7.60		7.64
Kaolinite	11.55	.43	14.12	12.29	14.83	17.15	15.62	7.94
Chlorite	.71	2.77	3.10		1.55	3.65		9.26
Enstatite				3.17	1.37		3.24	
Calcite			1.11	6.43	.88		9.39	
Magnesite				3.56			4.57	
Apatite		.39	.21	.94	.53		.14	.35
Gypsum					1.10		.22	
Ilmenite	1.33	.93	.26	1.12	1.37		.76	1.01
Hematite	2.76	3.70	3.25	2.97	2.71	4.02	2.17	
Magnetite					1.19		.64	
Water, etc	.85	8.51	.58		.10	3.12		7.03
Total	100.42	99.42	99.83	100.65	100.22	100.33	100.03	101.28

[a] A—Loess, Terre Haute, Ind. (*8, p. 562*).
B—Dustfall at Madison, Wis., Feb. 13, 1923.
C—Loess, Kansas City, Mo. (*1, p. 282*).
D—Loess, Mt. Vernon, Iowa (*4, p. 189*).
E—Loess, Dubuque, Iowa (*1, p. 282*).
F—Loess, Kansas City, Mo. (*5, p. 94*).
G—Loess, Galena, Ill. (*1, p. 282*).
H—Dustfall at Madison, Wis., March 19, 1920 (*12 p. 349*).

TABLE VIII.—*Number of spores per cubic centimeter of melted snow*

A. SAMPLE NO. 1

Kind of spore	Number of determinations						
	1	2	3	4	5	6	Average
Alternaria sp.	0.22	0	0	0	0	0	0.04
Fusarium sp.	0	0	0	0	.22	0	.04
Helminthosporium sp.	.22	.44	0	.22	0	.66	.26
Rusts, urediniospores	.22	.22	0	.22	0	.44	.19
Rusts, teliospores	0	0	0	0	0	0	0
Smuts	0	.22	.22	0	0	0	.07
Miscellaneous spores	.44	.88	.44	.44	.22	.88	.55
Mycelium	.66	.44	.66	.44	.44	1.10	.62

B. SAMPLE NO. 2

Alternaria sp.	0	0	0.22	0	0.22	.....	0.09
Helminthosporium sp.	0	0	.22	0	.66	.....	.18
Rusts, urediniospores	0	0	.44	.44	.22	.....	.22
Rusts, teliospores	0	0	0	0	0	.....	0
Miscellaneous spores	.44	.88	1.10	.44	.88	.....	.75
Mscellaneous mycelium	.44	.44	.66	.44	0	.....	.20

C. SAMPLE NO. 3

Kind of spore	Number of determinations					
	1	2	3	4	5	Average
Rusts, urediniospores	0.22	0	0	0	0	0.04
Miscellaneous spores	1.54	.88	.88	.66	1.32	1.06
Miscellaneous mycelium	.88	.44	0	.44	.44	.44

D. SAMPLE NO. 4[a]

Alternaria sp.	0	0.22	0	0	0	0.04
Helminthosporium sp.	.22	.22	0	0	0	.09
Rusts, urediniospores	.22	0	0	0	0	.04
Rusts, teliospores	0	0	0	.22	0	.04
Miscellaneous spores	.22	0	0	.22	.22	.13
Mycelium	0	.44	.22	0	0	.13

E. SAMPLE NO. 5[a]

Alternaria sp.	0	0	0	0	0.22	0.04
Helminthosporium sp.	0	0	0	0	0	0
Rusts, urediniospores	0	0	0	0	0	0
Rusts, teliospores	.22	0	0	0	0	.04
Miscellaneous spores	.44	0	.44	.44	0	.27
Mycelium	0	0	0	0	0	0

[a] Samples 4 and 5 contained too many mineral particles to count directly, therefore the melted snow was allowed to stand until the heavy particles settled out, when the counts were made on the decanted solution.

SUMMARY AND CONCLUSIONS

The present paper describes and discusses the third of four dustfalls known to have occurred in this country within the past six years. Two dustfalls have already been reported by the writers, (*11*, *12*), and analyses are under way for a report upon the fourth. The physical, chemical, mineral, and vegetal composition of the dustfall of February 13, 1923, is discussed in connection with that of other dustfalls of this country and abroad, and tabular analyses are presented.

Dustfalls are comparatively rare, and have always occurred in association with storms of late winter and early spring marked by unusually violent winds in the arid and semiarid regions of the plains, the Rocky Mountains, and the southern plateau of the United States.

The close similarity of composition of atmospheric dust to loess, and the divergence of such dust from other types of soils, suggest very strongly that the two are identical. The dustfalls of 1918 and 1920 showed on chemical analysis a higher content of soluble alkalies than is found in loess. Inasmush as the dust-bringing storms came in those instances from the arid southwest, it may be that they furnish the primitive material from which the loess has been formed. In 1923 the scene of deflating winds was closer at hand, and the coarser composition indicates that the atmospheric dust had been transported a slighter distance, perhaps from the region of the Missouri valley, where loess deposits are deepest.

The great depth of the deposits of loess, amounting to hundreds of feet in some places in the States of the Missouri Valley, compared with the present slow rate of deposition of atmospheric dust, indicates that dust-bearing storms were probably more frequent in the period when the loess was laid down. That could have been accomplished if the weather of the glacial period had been more continuously like our present February and March weather. At the same time there must have been extensive areas in the Southwest, uncovered by vegetation, from which the finer soil particles could be blown away by the wind. The fine size of the loess components indicates that they had been carried long distances by the wind, rather than obtained by deflation in the vicinity of deposit.

The presence of viable spores of plant disease in association with atmospheric dust which has been transported hundreds and even thousands of miles, indicates that such diseases may be rapidly spread over longer distances than plant pathologists have hitherto considered possible.

A peculiar psychological phenomenon associated with dustfalls is the fact that scarcely anyone notices them. Inquiries broadcasted during and after a dustfall elicit surprisingly few positive responses. But the complete study of such widespread phenomena as dustfalls demands more extensive attention. The writers will be glad to have the cooperation of other investigators in this field.

LITERATURE CITED

(1) CHAMBERLIN, T. C., AND SALISBURY, R. O.
1885. PRELIMINARY PAPER ON THE DRIFTLESS AREA OF THE UPPER MISSISSIPPI VALLEY. U. S. Geol. Surv. Ann. Rpt. (1884–85) 6: 199–322.

(2) HALL, E. C., AND OTHERS.
1921. SOIL SURVEY OF RINGGOLD COUNTY, IOWA. U. S. Dept. Agr. Bur. Soils, Field Oper. 1916, Rpt. 18: 1905–1929, illus.

(3) HAWKER, H. W., AND JOHNSON, H. W.
1919. SOIL SURVEY OF MUSCATINE COUNTY, IOWA. U. S. Dept. Agr. Bur. Soils, Field Oper. 1914, Rpt. 16: 1825–1884, illus.

(4) KNIGHT, N.
1902. ANALYSIS OF THE MOUNT VERNON LOESS. Amer. Geol. 29: 189.

(5) MCCOURT, W. A., ALBERTSON, M., AND BENNE, J. W.
1917. THE GEOLOGY OF JACKSON COUNTY. Mo. Bur. Geol. and Mines. [Rpts.] (ser. 2) v. 14, 158 p., illus.

(6) MARSHALL, P.
1903. DUST STORMS IN NEW ZEALAND. Nature 68: 223.

(7) PALMERI, P.
1901. SUL PULVISCOLI TELLURICI E COSMICI E LE SABBIE AFFRICANE. ANALISI E CONSIDERAZIONI. Rend. Accad. Sci. Fis. e Mat. [Naples] (ser. 3, v. 7) 40: 154–173.

(8) SCOVELL, J. T.
1897. GEOLOGY OF VIGO COUNTY, INDIANA. Ind. Dept. Geol. and Nat. Resources Ann. Rpt. (1896) 21: 507–576.

(9) SHAW, W. N.
1911. FORECASTING WEATHER, 1911. 380 p., New York.

(10) THORPE, T. E.
1903. "RED RAIN" AND THE DUST STORM OF FERUARY 22. Nature 68: 53–54, 222–223.

(11) WINCHELL, A. N., AND MILLER, E. R.
1918. THE DUSTFALL OF MARCH 9, 1918. Amer. Jour. Sci. (ser. 4, v. 46) 196: 599–609.

(12) ———
1922. THE GREAT DUSTFALL OF MARCH 19, 1920. Amer. Jour. Sci. (ser. 5, v. 3) 203: 349–364, illus.

PREPARASITIC STAGES IN THE LIFE HISTORY OF THE CATTLE HOOKWORM (BUSTOMUM PHLEBOTOMUM)[1]

By BENJAMIN SCHWARTZ

Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

The occurrence of hookworm infestation in cattle in the United States attracted considerable attention several years ago because of the supposed etiological relation of these parasites (*Bustomum phlebotomum*) to a disease of cattle commonly known as "salt sick." Following the report of Stiles (*14*),[2] who called attention to the occurrence of hookworms in cattle in Texas, Dawson (*5*) pointed out the resemblance between the clinical picture of hookworm anemia in man and "salt sick" in cattle, and in 1906 the same writer expressed a more definite opinion regarding the relation of cattle hookworms to "salt sick," which he characterized as "an acute or chronic parasitic disease manifested at first by low fever, diarrhea, loss of appetite; soon becoming chronic, with continuance of low fever, constipation, loss of appetite, progressive emaciation and pronounced anemia, which in many cases terminates fatally." While it has not been conclusively established that the condition in cattle commonly known as "salt sick" is actually due to hookworm infestation, the fact that cattle heavily infested with these parasites develop a progressive anemia, as shown by Dawson (*6*), has been confirmed by other observers, notably by Reisinger (*13*).

With a single exception, the published reports dealing with hookworm disease in cattle contain no information based on original investigations concerning the life history of *Bustomum phlebotomum*. This exception is a paper by Conradi and Barnett (*4*) which contains a very brief account of the development of the egg up to the infective stage, but lacks essential details. Since precise information concerning the preparasitic stages in the life history of strongyle parasites is generally useful in connection with control measures against these parasites, it is important that pertinent facts concerning the life cycle of cattle hookworms become available in order that such control measures may be devised. Hence the observations and experiments described in this paper were undertaken. As the work progressed some observations and experiments of a less practical nature were included in order to compare the behavior of cattle hookworm larvæ with those of other strongyle parasites.

MATERIAL AND METHODS OF INVESTIGATION

Specimens of hookworms from cattle, collected in the course of post-mortem examinations of the viscera, were washed several times in physiological salt solutions and then chopped with a pair of fine scissors to liberate the eggs from the uteri of the females. With the aid of a pipette chopped-up worm material was added to the surface of feces and charcoal mixtures in glass dishes and bottles (solid culture media) and to water and filtrate from boiled feces in staining jars and Petri dishes (liquid culture media).

The solid culture media were made up as follows: Sheep or cattle feces were boiled in water and filtered through ordinary filter paper. Sufficient charcoal was added to the filtrate to make a thick paste, which was spread out in Petri dishes, wide-mouth bottles, and other glass containers, care being taken to smooth down the surface of the medium and to keep the latter moist by adding water whenever necessary in order to make up the loss due to evaporation. When diluted with water sufficient to make a transparent medium, the filtrate from the boiled feces was found to be an excellent culture medium for the examination of contents from day to day. A thin layer of the liquid was added to the glass containers, thus affording a supply of oxygen, which is requisite to rapid development. Culture media were kept at room temperature (70°

[1] Received for publication Apr. 10, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 458.

to 80° F.) and examinations were made by mounting the glass vessels containing liquid media directly on the stage of the microscope for general observation, and resorting to isolation of larvæ whenever detailed observations on structure or behavior were desirable.

Inasmuch as not all eggs hatch at the same time, because of variation in rate of development and various other factors, newly hatched larvæ were isolated and mounted on cover glasses inverted over hollow ground slides or over ordinary slides to which temporary chambers were sealed. Thus the changes undergone by the larvæ could be followed very readily without such confusion as would result from having larvæ at various stages of development in the same dish. If precautions are taken to secure good-sized drops, and if hanging-drop preparations are properly sealed, the changes in the larvæ from the time of hatching to the infective stage may be followed without adding additional fluid, thus avoiding the need of removing the cover glass at various intervals. In one series of observations eggs and larvæ were secured from day to day from solid culture media and their development was compared with that of eggs and larvæ in liquid media. No noteworthy differences in rapidity of development were found; therefore observation on the rapidity of development in subsequent series of cultures was limited to those made on liquid media.

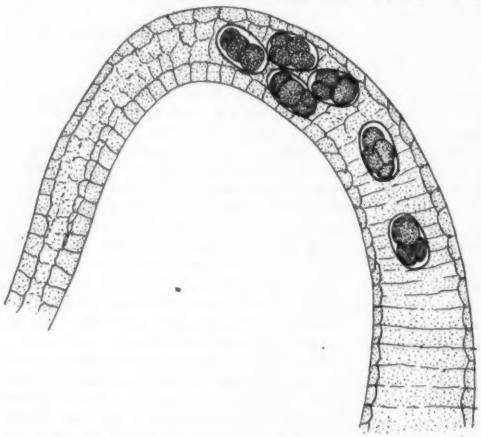


FIG. 1.—Portion of uterus containing segmented eggs

DESCRIPTION OF THE EGG

The egg of *Bustomum phlebotomum*, in common with eggs of other strongyles, is thin-shelled, elliptical in shape, and exhibits considerable range in size (52 to 106 μ in length, by 43 to 60 μ in width). In a series of measurements involving more than 100 eggs only 10 per cent of the eggs exceeded a length of 100 μ and only about 5 per cent were shorter than 75 μ. The majority of eggs measured were from 85 to 95 μ long and slightly over 50 μ wide. No definite correlation was found between length and width of eggs. Some long eggs are comparatively narrow and some present an almost spherical appearance.

DEVELOPMENT OF THE EGG

Conradi and Barnett (*4*) state that before the egg is passed from the body of the host it is in the mulberry stage. These writers also note that partial incubation of the egg in the intestine of the host seems to be important, since eggs that were taken from the host's intestine and thus missed the partial incubation period in the intestine showed a heavier mortality than eggs collected from feces. The present writer noted that eggs begin to segment in the uterus of the female parasite, as shown in Figure 1. Eggs obtained as a result of chopping freshly collected worms are in the one-, two-, and four-cell stages, and rarely in the eight-cell stage. At room temperature (70° to 80° F.) the development of such eggs takes place rather slowly. After 24 hours of incubation it was noted that comparatively few eggs had reached the mulberry stage; and after 72 hours of incubation comparatively few embryonated eggs, with embryos moving in the shells, were found. Ninety-six-hour cultures usually showed free larvæ as well as embryonated eggs, the latter hatching in the course of a few hours. According to Conradi and Barnett (*4*), at a temperature of 48° to 60° it required 31 days for the eggs to hatch. These writers also report that eggs obtained from feces on February 26 hatched on February 28; but they do not give the temperature at which the eggs were incubated. According to observations by the present writer, eggs at various stages of development undergo disintegration in culture media not only because of the presence of bacteria in

cultures, but also because of other factors probably present within the egg as well as in the surrounding medium.

PREINFECTIVE LARVÆ

The first-stage larva exhibits lively movements, twisting its body in typical nematode fashion. Several first-stage larvæ were kept under observation about seven hours, during which period they were incessantly active, moving about with vigorous flexures of the anterior portion of the body aided by the propelling movements of the long, slender tail.

These larvæ are commonly from 420 to 450 μ long, or somewhat longer, by 20 to 25 μ wide. The first third of the body is the broadest, the remaining portion tapering gradually and terminating in a slender tail. Structurally, the organism is relatively simple, its most conspicuous organs being an alimentary canal consisting of an esophagus with a terminal bulb and a straight intestine (this being densely granular in contrast to the less granular esophageal region), and a genital primordium.

About 24 hours after hatching, first-stage larvæ became very sluggish, a physiological condition (lethargus) which precedes moulting. Examination of such sluggish larvæ with high magnification generally showed a separation of the cuticle in the cephalic extremity, revealing a newly formed cuticle underneath the old one.

The span of life of the second-stage larvæ is comparatively brief, since 18 hours after the first lethargus was observed the second lethargus was found to be in progress. The second-stage larva shows a slight increase in size (490 by 25 μ) and is less coarsely granular than the first-stage larva. Before its quiescent stage preparatory to the final molt it shows moderate activity, less marked, however, than that of the first-stage larva.

Although in a 6-day-old culture several quiescent larvæ undergoing the final molt were found, third-stage larvæ were generally not observed before the eighth day after making the culture. The second lethargus lasts at least 24 hours.

The duration of the preinfective stages in the life of the larvæ may be increased by subjecting them to low temperatures, which retard their development. Thus first-stage larvæ were prevented from further development for a period of 10 days by being kept in water in a refrigerator at a temperature of 8° C. While a number of larvæ degenerated during that period, others were still alive and showed no changes of structure or other evidence of having molted. Meanwhile larvæ from the same lot at room temperature under-

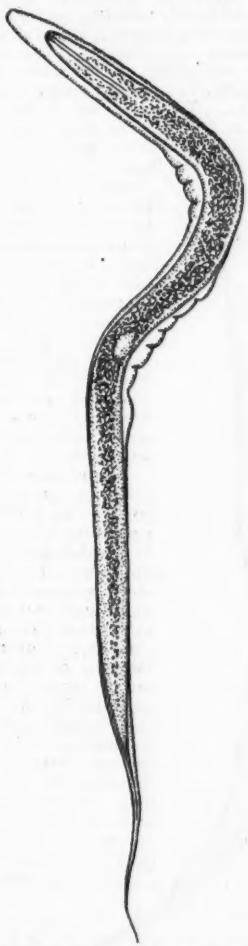


FIG. 2.—Outline drawing of infective larva from solid culture medium showing protective sheath

went their normal course of development.

INFECTIVE LARVÆ

In rich 8-day-old cultures third-stage larvæ were found in abundance. These organisms are readily recognized by their greater transparency, which in-

creases as they are kept alive in cultures. The earlier-stage coarser granules practically disappear and the larvæ also retract within the shells, showing quite distinctly the protective sheath, which is usually wrinkled (fig. 2).

The infective larvæ are generally from 500 to 540 μ long by 20 to 27 μ wide, although infective larvæ slightly less than 500 μ long were found on several occasions. The distance from the cephalic extremity to the base of the esophagus was found to vary from 125 to 145 μ. The diameter of the esophagus at the base is 10 μ. The nerve ring has a diameter of 5 μ and is usually situated 60 μ from the cephalic extremity. In one specimen the distance was found to be 78 μ, while in another specimen it was only 50 μ. The distance from the anterior extremity to the genital primordium is slightly in excess of 200 μ. The genital primordium is 10 μ long by 5 μ wide. The tail corresponds roughly to the terminal sixth of the total body length (fig. 4).

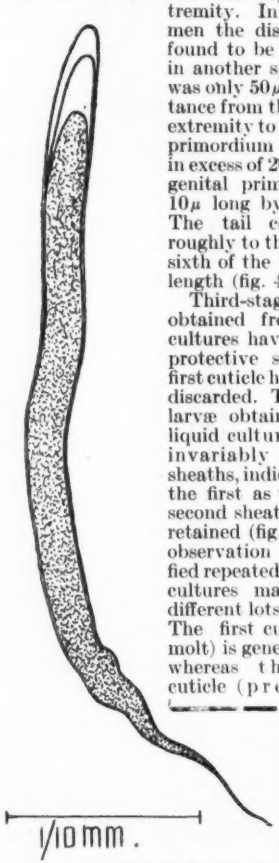


FIG. 3.—Outline drawing of infective larva from liquid culture medium showing two sheaths

Third-stage larvæ obtained from solid cultures have only the protective sheath, the first cuticle having been discarded. Third-stage larvæ obtained from liquid cultures almost invariably show two sheaths, indicating that the first as well as the second sheath has been retained (fig. 3). This observation was verified repeatedly in liquid cultures made from different lots of worms. The first cuticle (first molt) is generally rigid, whereas the second cuticle (protective sheath) is flexible and is often wrinkled.

Larvæ with two sheaths from liquid cultures were kept under observation in cover-glass preparations, and it was noted that as the slide became almost dry certain larvæ that were in contact with some solid object discarded the first sheath. This observation was repeated a number of times and indicates that contact with a solid object is necessary to exsheathing, and also probably that a liquid medium is inimical to the completion of the ecdysis. According to Augustine (1), the first ecdysis in *Ancylostoma duodenale* is due to the fact that the larva has increased in size considerably and thus bursts the tightly surrounding sheath. The slight difference in size between first- and second-stage larvæ of *Bustomum phlebotomum* renders other mechanical stimuli necessary for the completion of the first ecdysis, and in the absence of these stimuli the first cuticle is retained.

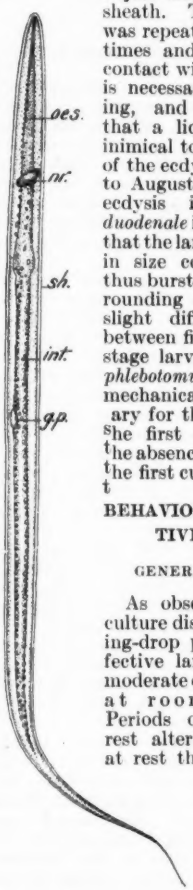


FIG. 4.—Infective larva. oes., oesophagus; n. r., nerve ring; sh., sheath; int., intestine; g. p., genital primordium

BEHAVIOR OF INFECTIVE LARVÆ

GENERAL BEHAVIOR

As observed in liquid-culture dishes and in hanging-drop preparations, infective larvæ show but a moderate degree of activity at room temperature. Periods of activity and rest alternate, and while at rest the larva is either straight and rigid or somewhat coiled and rigid. The protective sheath is generally visible, because the body is always more or less retracted within the sheath. Quiescent third-stage larvæ usually become active when disturbed. A transfer from a culture dish to a slide stimulates them to activity involving rapid movements, which gradually become less intense and ultimately cease entirely.

On slide preparations it was noted that the larvæ commonly exhibit a tendency to come to rest when in contact with some solid object such as débris carried over from the culture medium. Larvæ with the anterior

portion of the body concealed under some solid object and the rest of the body straight and rigid are usually seen in culture dishes and cover-glass preparations.

Infective larvæ appear generally more tolerant of an unfavorable environment than are preinfective larvæ. Preinfective larvæ are easily killed in culture dishes when bacteria are present in large numbers, the heaviest mortality occurring during the lethargus. But infective larvæ were found in culture media containing massive bacterial growths. While preinfective larvæ can maintain their vitality at a low temperature, they frequently degenerate in a refrigerator, whereas this phenomenon was not observed in infective larvæ, which were kept at 8° C. in water without showing any signs of degeneration. Infective larvæ were also frozen solid for about 15 hours outside of a window, and after being thawed they gradually resumed their normal shape and became active, showing what appeared to be a complete recovery in about 5 hours, whereas preinfective larvæ are easily killed by cold, according to Conradi and Barnett (*4*). Ransom (*12*) showed that the preinfective larvæ of *Haemonchus contortus* offer comparatively little resistance to freezing, although infective larvæ could be frozen and successively thawed out many times.

REACTION TO HEAT

Under the influence of heat the larvæ exhibit very lively movements. Thus, if the point of a needle heated in a flame or the heated end of a glass rod is brought into contact with the undersurface of a glass slide containing larvæ, they become very active and orient themselves toward the source of the heat, moving very rapidly in that direction. The orientation of the larvæ with reference to the source of heat is unfailing, and the writer took advantage of this reaction to concentrate larvæ in the center of a dish in which comparatively few larvæ were present. Holding a hot glass rod against the outer surface of the bottom of a Petri dish caused the larvæ to collect in a circumference around the point to which heat was applied, approaching thither from all directions.

While heat is an unfailing source of stimulation to the larvæ, it was noted that larvæ which collected near the edge of a drop of water on a slide, a tendency which they frequently exhibit, do not orient themselves toward the source of heat but merely exhibit feverish activity by vigorous and spasmodic movements of the body, without turning the cephalic extremity which continues to be in contact with the periphery of the drop.

If too much heat is applied the larvæ cease their movements before they reach the source of heat, coiling up instead and resuming their activities as the heat diminishes.

Khalil (*9*) showed that the infective larvæ of a number of nematodes are positively thermotropic. He obtained positive results with *Ancylostoma duodenale*, *Necator americanus*, *Ancylostoma ceylanicum*, *Strongyloides stercoralis*, *Galoncus perniciosus*, and *Trichostrongylus douglasi*, and negative results with *Haemonchus contortus*. As *Haemonchus contortus* is not a skin penetrator, Khalil concluded that only skin penetrators are positively thermotropic. Recently Cameron (*3*) showed that *Monodontus trigonocephalus* is positively thermotropic, although the larvæ do not penetrate the skin under experimental conditions identical with those under which *Ancylostoma ceylanicum* penetrates it. As will be shown later, *Bustomum phlebotomum* does not penetrate the skin under experimental conditions, thus affording further evidence that there is no correlation between positive thermotropism and ability of larvæ to penetrate the skin. In this connection it may be of interest to note that the larvæ of Nematodirus are negatively thermotropic according to Cameron's observations (*3*).

REACTION TO STAIN

The reaction of the infective larvæ to stain was studied by allowing a 1 per cent solution of basic fuchsin to run in under cover-glass preparations containing larvæ. As the stain runs in the larvæ are active at first; but that this activity is independent of the stain and is due to the current can be readily shown by running in water under the cover-glass. As the larvæ become enveloped in stain they generally become quiescent but do not absorb the stain. Larvæ were kept under observation for several hours (6 hours in one case and 3 hours in two other cases), but no penetration of the stain into the tissues of the larvæ was observed. The stain readily penetrates the sheath, as is seen distinctly whenever a larva retracts within the sheath, but the writer found no evidence of penetration of the stain beyond that point. The larvæ remained in the stain for hours without loss of vitality. Their vitality while quiescent can be demonstrated (1) by running water

under the cover glass, the current thus set up acting as a stimulant to activity, (2) by applying heat, (3) by watching for spontaneous signs of activity, which usually occur at variable intervals. Throughout this series of observations, involving a number of larvæ, one larva showed evidence of having absorbed some stain, but it had lost its vitality and showed no response to stimulation.

Cameron (*3*) states that the larvæ of *Monodontus trigonocephalus* when treated with fuchsin absorb the stain rapidly and die, in contrast to the behavior of the larvæ of *Ancylostoma duodenale*, which fail to absorb the stain and escape from their sheaths, which alone become stained, as shown by Looss (*10*) and by other investigators. Cameron also states: "Goodey (*8*) showed that this was also true for Necator larvæ; but that Haemonchus and Graphidium which do not penetrate the skin, absorbed the stain and died." He further states that all skin penetrators that have been tested exsheathed, whereas nonskin penetrators have not exsheathed but died when treated with stain. In Goodey's paper to which Cameron refers the only statements concerning reaction of nematode larvæ to stains are the following: "I found that *N. americanus* larvæ come out of their sheaths, as found by Herman and confirmed by Looss, when the drop containing the larvæ and stain is covered with a cover slip. *G. strigosum* and *T. retortaeformis* larvæ did not exsheath." Nowhere in Goodey's paper are there any references to the behavior of Haemonchus larvæ in the presence of stain or any reference to the fact that the larvæ of Graphidium and Trichostrongylus were killed by stains (methyl green and fuchsin).

In the present writer's experiments Bustomum larvæ did not exsheath in the presence of fuchsin, nor coil up and die. As will be shown later, there is no necessary correlation between the reaction of larvæ to stains and their ability to penetrate skin.

EFFECTS OF DRYING

According to Conradi and Barnett (*4*), infective larvæ of *Bustomum phlebotomum* are resistant to drought, but according to the writer's observations infective larvæ of *Bustomum phlebotomum* are not resistant to drying. Slide preparations with and without cover glasses containing larvæ were allowed to dry at room temperature for periods varying from one to several hours. After being moistened it was found that the larvæ were retracted in their sheaths, and although the retracted larvæ absorbed water, as a result of which they gradually filled out the empty spaces in the cuticle, they did not regain their vitality. These observations were made during the winter months in a steam-heated laboratory having a very low humidity content, and were repeated a number of times with similar results. Although the larvæ are not resistant to drying, they can maintain their vitality for a long time in the presence of a small amount of moisture. Thus a solid-culture medium in a covered Petri dish to which water had not been added for about three weeks and which had the appearance of being dry yielded larvæ many of which had retained their vitality and exhibited lively movements after being moistened.

Nematode larvæ exhibit considerable variation in resistance to drying. Ransom (*12*) showed that the larvæ of *Haemonchus contortus* are highly resistant to drying, and that they can be revived after 35 days' exposure to drying. Looss (*10*) found that the larvæ of *Ancylostoma duodenale* shrivel up and die when the moisture evaporates from their bodies. Boulenger (*2*) showed that Nematodirus larvæ are resistant to drying, and this observation has been confirmed by Cameron (*3*).

Goodey (*8*) confirms Looss's observation for *Necator americanus* and states: "It seems a natural inference to draw therefore that Necator and Ancylostoma seek the protection afforded by penetration into the skin because if they remained outside and became dry they would perish." The same writer found that the larvæ of *Graphidium strigosum* and of *Trichostrongylus retortaeformis* can withstand ordinary desiccation at room temperature for a few days and revive on the addition of water, behaving in this respect like the larvæ of *Haemonchus contortus* as shown by Ransom (*12*) and confirmed by Veglia (*15*). Cameron (*3*) found that the larvæ of *Monodontus trigonocephalus* offer little resistance to drying, and since these larvæ are not skin penetrators, it is unsafe to attempt to correlate susceptibility to drying with the habit of boring through the skin. The present writer's observations with reference to the behavior of the larvæ of *Bustomum phlebotomum*, which do not penetrate the skin under experimental conditions, lend no confirmation to the view that nonskin penetrators are resistant to drying.

REACTION TO LIGHT

The ensheathed larvæ of *Bustomum phlebotomum* are positively phototropic, as the following observations will show.

In a 14-day-old culture on a charcoal-and-feces mixture larvæ were found along the walls of the bottle facing the light of a north window, but none was found along the walls of the bottle facing the room. In a 10-day-old culture which was kept in a dark place no larvæ were found along the walls of the bottle, whereas in a culture made from the same lot of worms but kept near the window larvæ were found crawling up the walls of the bottle facing the light. Similar observations made at various times on larvæ in cultures indicate that they respond positively to light by collecting in parts of culture dishes exposed to light and by being absent from shaded portions.

In their light reactions the larvæ of *Bustomum phlebotomum* resemble the larvæ of Monodontus (Cameron, *3*) and *Haemonchus contortus* (Veglia, *15*).

REACTION TO GRAVITY

Conradi and Barnett (*4*) noted that the larvæ of *Bustomum phlebotomum* crawl up the walls of culture jars. The present writer's observations bear out this point. Whether this upward movement of the larvæ is a negative geotropism or whether it is merely an expression of their positive phototropism is not clear from the data at hand. The tendency of the larvæ to climb up the walls of culture dishes, and their presumably similar tendency to climb up blades of grass and other objects in nature, is doubtless an adaptation to secure an advantageous position in order to complete their life cycle. Ransom (*12*) pointed out the adaptation of the upward migration of Haemonchus and other strongyle larvæ to the feeding habits of ruminants, which favors the ingestion of the larvæ by the host. Similar tendencies have been observed in most larvæ belonging to the superfamily Strongyloidea, Syngamus, according to Ortlepp (*11*), and Monodontus, according to Cameron (*3*), being exceptions.

EXPERIMENTS ON SKIN PENETRATION

In 1919 infective larvæ of *Bustomum phlebotomum* were placed on the skin of guinea pigs, the area on which the larvæ were placed having been previously shaved. In the course of several experiments no local skin reaction was observed in these experimental animals, and intact larvæ were recovered from the skin. Later, experiments were made in accordance with the method devised by Goodey (*7*). Skin from a 7-day-old rat was stretched on a thin sheet of cork in the center of which was a hole about an inch in diameter, and the cork was floated in physiological salt solution in a glass dish kept in an incubator at a temperature of 37°. A drop of water containing a number of larvæ was placed on the skin. Repeated examinations showed the larvæ on the surface of the skin. The larvæ were sucked up in a pipette and examined from time to time on a glass slide. There was no evidence of their having molted. After the experiment had been in progress several hours the drop of water on the rat's skin containing the larvæ was allowed to evaporate. A drop of distilled water was added to the skin and the larvæ were sucked up in a fine pipette and examined microscopically. They were nearly all active. The skin was then fixed in 70 per cent alcohol and cleared in lactophenol. Microscopic examination of the cleared skin failed to show any evidence of penetration by the larvæ.

Cameron (*3*) has shown that the larvæ of a related hookworm (*Monodontus trigonocephalus*) do not penetrate the skin under experimental conditions. Since well-known skin penetrators (Ancylostoma and Necator) penetrate the skin under similar experimental conditions, this method may be considered a reliable test of the ability of nematode larvæ to bore into the skin.

Assuming, therefore, that the results obtained by Cameron (*3*) with the larvæ of Monodontus and the results obtained by the present writer with Bustomum give reliable information as regards the inability of the larvæ of these genera to penetrate the skin, it is evident from the data and discussion presented in the foregoing pages that attempts of various investigators to establish correlations between behavior of nematode larvæ and their probable mode of entry into the host is untenable.

SUMMARY

(1) Under laboratory conditions at a temperature of 70° to 80° F. the eggs of *Bustomum phlebotomum* hatch in about 96 hours. The first-stage larvæ are found in lethargus 24 hours after hatching, and 24 hours later the second lethargus is in progress. After a second lethargus, which lasts at least 24 hours, third-stage larvæ emerge,

the complete cycle of development up to this stage requiring a minimum of 7 days.

(2) In liquid cultures both cuticles are usually retained by the larvæ, whereas in solid cultures the first cuticle is cast off. Observations on the loss of the first skin by infective larvæ from liquid cultures indicate that contact with some solid object is necessary for exsheathing, and that in a liquid medium this process does not ordinarily take place.

(3) Infective larvæ are only moderately active at room temperature, but may be readily stimulated to activity by mechanical factors. These larvæ appear more resistant to an unfavorable environment than do preinfective larvæ.

(4) Infective larvæ are positively thermotropic and orient themselves in such a way that the cephalic extremity becomes directed to the source of heat, toward which the larvæ swim rapidly.

(5) In the presence of a solution of fuchsin the larvæ behave in a manner unlike that observed heretofore in other nematode larvæ. They neither absorb the stain and die nor do they exsheath, but remain alive and apparently unaffected by the stain, which after several hours' contact with the larvæ does not penetrate beyond the sheath or sheaths.

(6) The infective larvæ succumb to desiccation, but can maintain their vitality under conditions which afford a slight amount of moisture.

(7) The infective larvæ are positively phototropic, collecting in the lightest portion of the culture medium. They also crawl up the walls of culture bottles, but whether the latter is to be interpreted as a negative geotropism or is due to the positive phototropism of the larvæ is not certain.

(8) Under experimental conditions the larvæ show no tendency to penetrate the skin.

(9) Attempts made by various writers to correlate the behavior of various strongyle larvæ with their skin-penetrating habits appear to be untenable from the data presented in this paper and from data obtained by others.

LITERATURE CITED

(1) AUGUSTINE, D. L.
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XIX. OBSERVATIONS ON THE COMPLETION OF THE SECOND ECDYSIS OF NECATOR AMERICANUS. Amer. Jour. Hyg. 3: 280–295, illus.

(2) BOULENGER, C. L.
1915. THE LIFE HISTORY OF NEMATODIRUS FILICOLLIS RUD., A NEMATODE PARASITE OF THE SHEEP'S INTESTINE. Parasitology 8: 133–155, illus.

(3) CAMERON, T. W. M.
1923. ON THE BIOLOGY OF THE INFECTIVE LARVA OF MONODONTUS TRIGONOCEPHALUS (RUD.) OF SHEEP. Jour. Helminthol. 1: 205–214.

(4) CONRADI, A. F., AND BARNETT, E.
1908. HOOKWORM DISEASE OF CATTLE (UNCINARIASIS). S. C. Agr. Exp. Sta. Bul. 137, 23 p., illus.

(5) DAWSON, C. F.
1903. HOOKWORMS IN CATTLE. Fla. Agr. Exp. Sta. Press Bul. 36, 3 p.

(6) ——
1906. SALT SICK (BOVINE UNCINARIASIS). Fla. Agr. Exp. Sta. Bul. 86, 14 p.

(7) GOODEY, T.
1922. A SIMPLE METHOD OF EXPERIMENTATION FOR SKIN INFECTION WITH HOOKWORM LARVÆ. Proc. Roy. Soc. Med. (Sect. Trop. Dis. and Parasitol.) 15(4): 19–20.

(8) ——
1922. OBSERVATIONS ON THE ENSHEATHED LARVÆ OF SOME PARASITIC NEMATODES. Ann. Appl. Biol. 9: 33–48, illus.

(9) KHALIL, M.
1922. THERMOTROPISM IN ANKYLOSTOME LARVÆ. Proc. Roy. Soc. Med. (Sect. Trop. Dis. and Parasitol.) 15: 16–18.

(10) LOOSS, A.
1911. THE ANATOMY AND LIFE HISTORY OF AGCHYLOSTOMA DUODENALE DUB. A MONOGRAPH. PART 2. THE DEVELOPMENT IN THE FREE STATE. Egypt Ministry Ed., Rec. School Med. 4: 159–613, illus.

(11) ORTLEPP, R. J.
1923. THE LIFE-HISTORY OF SYNGAMUS TRACHEALIS (MONTAGU) V. SIEBOLD, THE GAPE-WORM OF CHICKENS. Jour. Helminthol. 1: 119–140, illus.

(12) RANSOM, B. H.
1906. THE LIFE HISTORY OF THE TWISTED WIRE WORM (HAEMONCHUS CONTORTUS) OF SHEEP AND OTHER RUMINANTS. U. S. Dept. Agr. Bur. Anim. Indus. Circ. 93, 7 p., illus.

(13) REISINGER, L.
1916. BEITRAG ZUR ANKYLOSTOMIASIS DES RINDES. Wiener Tierärztl. Monatsschr. 3: 467–487, illus.

(14) STILES, C. W.
1901. VERMINOUS DISEASES OF CATTLE, SHEEP, AND GOATS IN TEXAS. U. S. Dept. Agr. Bur. Anim. Indus. Ann. Rpt. (1900) 17: 356–379.

(15) VEGLIA, F.
1915. THE ANATOMY AND LIFE-HISTORY OF THE HAEMONCHUS CONTORTUS (RUD.). Union South Africa Dept. Agr. Rpt. Director Vet. Research 3/4: 347–500, illus.

A MYCORRHIZAL FUNGUS IN THE ROOTS OF LEGUMES AND SOME OTHER PLANTS[1]

By Fred Reuel Jones

Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

During an investigation of a fungus parasite of the roots of peas, it became necessary in the spring of 1922 to examine under the microscope fresh razor sections of rootlets of a great number of plants in different stages of development. In the course of this work it was found that very many of the rootlets of plants which were considered altogether normal were extensively invaded by a characteristic fungus which entered a few rootlets when the plant was small and had traversed at least half of the root system by the time the plant reached full bloom. The fungus was restricted to the primary cortex of the root, and usually indicated its presence by the straw-yellow color that the root assumed. When the pea plant was mature, all of the roots were usually yellow and thoroughly invaded by the fungus. This uniform and widespread infestation of pea roots indicated that the fungus must be very thoroughly distributed in the soil, and suggested the examination of roots of other legumes to learn whether they, too, might be hosts of this fungus. It was found that the roots of clover, alfalfa, sweet pea, and some other legumes were as thoroughly infested by this fungus growth as were those of peas.

The root-inhabiting fungus thus found so abundant in the smaller roots of these common and much studied legumes is so coarse in character, has such distinctive structures, and produces such conspicuous discoloration of a portion of the invaded tissue as seen under the microscope, that it is somewhat surprising to find that its presence is not a matter of common knowledge among botanists. The fungus itself, or very similar fungi, have long been known in the roots of some other plants under the name of mycorrhizal fungi, and recently several studies of these root parasites by botanists and pathologists have added many details to our knowledge of their development and have extended the list of plants in which they occur. But the list appears to contain only two species among the Leguminosae.[2]

Since the fungus is clearly parasitic in character, occurring abundantly in all or nearly all plants of the species infested, and producing in the invaded tissue unmistakable local pathological conditions, it became necessary to distinguish between the results of its invasion and those following the attack of more aggressive parasites which were under investigation. This study, which began with the roots of peas, was soon extended to the roots of other legumes, and has produced evidence suggesting that this fungus infestation may influence profoundly the development of some of our common legumes, at least when they are grown under certain environmental conditions. It is the purpose of this paper to record more fully than in a previous note (*3*)[3] the list of plants in the roots of which this fungus is found, to describe the local effect it produces, and to indicate the modification in the development of some of the infested plants to which this parasitism may contribute.

It may be unnecessary to add that the term mycorrhizal fungus, in its original coinage and as used in some recent papers dealing with this type of parasitism, does not carry with it any implication in advance of proof that the fungus is of any benefit to the plant invaded. In fact, it seems to be generally recognized that among the numerous observed examples of root invasions which have been designated by this term, many will undoubtedly be found to be wholly disadvantageous to the host and are properly within the plant pathologist's field of study.

[1] Received for publication May 28, 1924—issued January, 1925.

[2] Since this was written Peyronel has published a host list that includes many of the common cultivated legumes. Peyronel, B., prime ricerche sulle micorize endotrofiche e sulla micoflora radicicola normale delle fanerogame. Riv. Biol. 5: 463–485, illus., 1923; 6: 17–53, illus., 1924.

[3] Reference is made by number (italic) to "Literature cited," p. 470.

The literature on the type of mycorrhizal relation considered in this paper has been reviewed in recent papers by Magrou, Peyronel, and Demeter, cited elsewhere. It will suffice to note here that the first description of a mycorrhizal fungus in a plant belonging to the Leguminosae appears to have been made by Janse (*2*) in *Pithecolobium montanum*. Magrou (*4*) has given an excellent description of a similar or identical fungus in *Orobus* (*Lathyrus*) *tuberosus*. Doctor Magrou has exchanged stained preparations with the writer, and he states in personal correspondence that the fungus in the roots of the four legumes sent him appears identical with that which he found in *Orobus*. The writer concurs in this opinion. Peyronel (*5*) has recently published an abstract of a forthcoming paper recording this type of mycorrhizal invasion in a number of plants, and he describes what he believes to be the method of germination of the so-called vesicles. Demeter (*1*) has found the same type of mycorrhizal fungus in roots of *Vinca minor* and some related plants. His attempts to secure it in pure culture have yielded the first success that has been reported. From its cultural characters Demeter designates the fungus *Rhizoctonia apocynacearum*, without giving a formal description; but his attempts to infect plants with this culture did not satisfactorily reproduce the condition found in the field. In view of these unsatisfactory inoculation experiments and the obviously great differences between the fungus described in culture and that found in the plant, Demeter's conclusions need confirmation.

DISTRIBUTION OF THE MYCORRHIZAL FUNGUS IN THE ROOTS OF LEGUMINOUS PLANTS

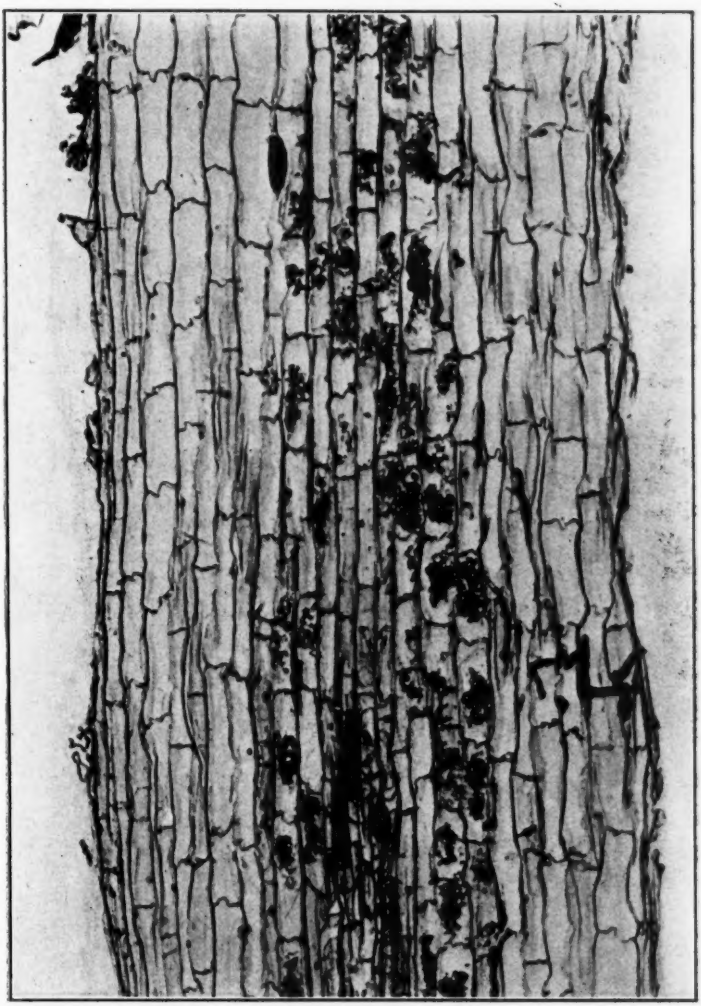
The mycorrhizal fungus of legumes, like that of similar character in other plants, is found only in the cortex of roots. In annuals such as peas and sweet peas it does not enter the cortex of the base of the stem, which is very rootlike in structure. In fact, the cortex of the taproot seems to be quite resistant to it. It develops most abundantly in the small lateral roots which produce little or no secondary thickening. In biennials and perennials such as clover and alfalfa the cortex of the taproot is sloughed off as soon as secondary growth increases the diameter of the root. Therefore the fungus is not commonly found in taproots or even in the large lateral roots. The small laterals and terminal branches of all roots in older plants produce little or no secondary thickening, and the primary cortex persists during the life of the roots. This cortex is readily penetrated and in it the fungus develops extensively. All the above-named plants have a comparatively thick cortex which occupies from two-thirds to three-fourths of the entire diameter, and in these hosts (Pls. 1, 2) the fungus develops far more abundantly than in the roots of beans and soybeans, which have comparatively thin cortical layers. Thus the thickness and character of the tissue outside the endodermis seem to determine the extent to which the plant may harbor the fungus. Perhaps the large intercellular spaces in the cortical tissue, which permit easy passage of the hyphae along the root, is the character to which the fungus has become adapted.

DISCOLORATION OF ROOTS BY THE MYCORRHIZAL FUNGUS

When the cortex of the root has become thoroughly invaded, the condition is usually indicated by a yellow or greenish-yellow discoloration, sometimes accompanied by a slightly water-soaked, translucent appearance. This discoloration is due, as will be shown later, to the color of the contents of the deeper cells, and to this source is due the characteristic appearance distinguishing it from those discolorations which arise from changes in the walls of the outer cells. In advanced stages the walls of the outer cells may turn yellow as the roots begin to disintegrate or from some other cause, whereupon the characteristic appearance is lost. The larger part of the discoloration in the roots of legumes which have been examined seems to be due to this fungus. The color does not indicate the extent to which invasion has progressed; at times it will be found to have advanced far beyond the discolored region. In all doubtful cases, the presence of the fungus can readily be determined by microscopic examination of sections of the root.

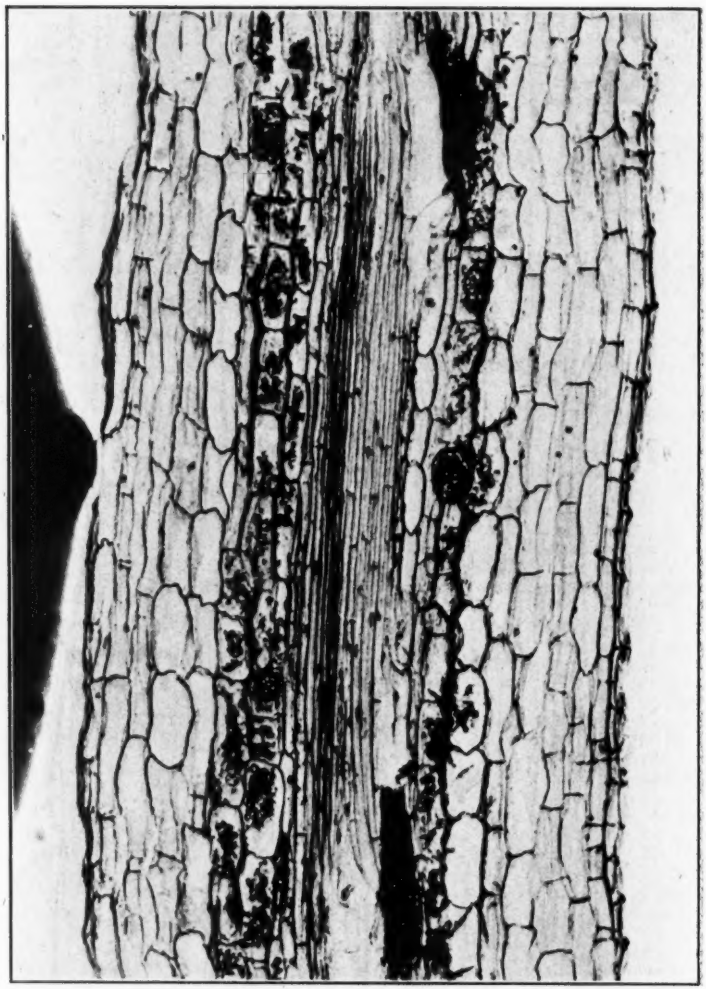
PATHOLOGICAL HISTOLOGY

An examination of free-hand longitudinal sections from fresh roots which are yellow and water-soaked will show at once where the color is located. It is usually limited to the cell sap of the cortical cells just outside the endodermis where its greenish-yellow or olive-green character is very striking in thick sections. This color soon diffuses from the cut cells of razor sections mounted in water, and is more slowly lost from



A Mycorrhizal Fungus in the Roots of Legumes Plate 1

Mycorrhizal fungus in a rootlet of red clover. Longitudinal tangential section including a few endodermal cells at bottom of plate. The penetration of the fungus is shown at the right margin of the root. The dark masses of irregular outline in the cells at the center of the section are arbuscles. A few fragments of longitudinal hyphae are shown between the cells. The dark elliptical body between two cells near the top of the plate is a small vesicle.



A Mycorrhizal Fungus in the Roots of Legumes Plate 2

Mycorrhizal fungus in a rootlet of red clover. A median longitudina lsection showing the fungus outside of the endodermis. The intercellular mycelium appears as dark lines. A vesicle is shown at the right of the center of the section.

entire roots which are kept submerged in water. The intercellular spaces of the discolored cells are more or less filled with a liquid, this giving rise to the water-soaked appearance of the tissue. The cells with discolored contents usually contain large, somewhat amorphous masses of uncolored material, the haustoria of the fungus (Pl. 1), which are sometimes even more conspicuous than the color, and these are unmistakable evidence of the presence of the fungus strands, which are sometimes difficult to discern in longitudinal sections. A cross section of an infected root shows more clearly the distribution of the strands from which the haustoria originate (fig. 1).[4] Usually they occur

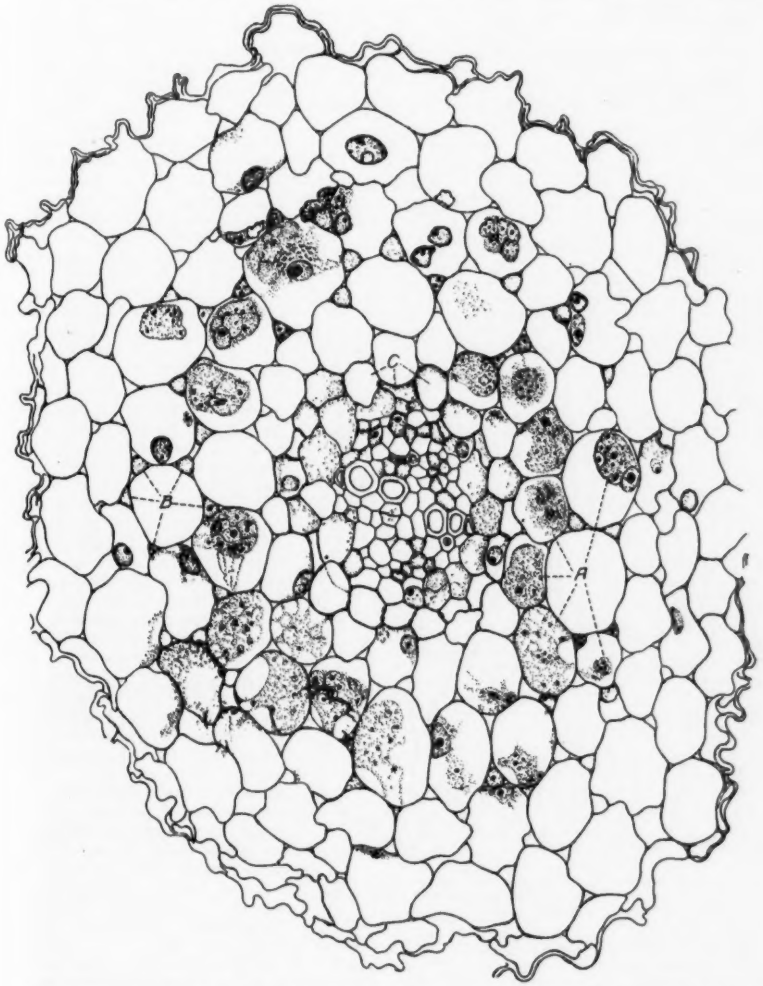


FIG. 1.—Transverse section of a small rootlet of *Trifolium pratense* containing the mycorrhizal fungus. A.—Degenerating haustoria. B.—Strands of intercellular mycelium, of which about 35 may be counted here. C.—Endodermal cells.

[4] The writer is indebted to Dr. Charles Drechsler for the drawing of all text figures.

only locally in the cortex, but are frequently distributed throughout its radial section. The fact that the tissue is not immediately destroyed indicates that a high degree of adaptation has been established.

DESCRIPTION OF THE FUNGUS

The mycorrhizal fungus in roots of legumes has been described and illustrated so well by Janse, Magrou, and Peyronel that only the more important morphological characters will be described here. The characteristic structures of the fungus have been designated mycelium, arbuscles, and vesicles. The arbuscles are, as Peyronel recognized, haustorial structures, comparable to those produced by some of the Peronosporaceae, though somewhat more elaborate in structure. Since there is no apparent advantage in the use of a distinctive term, they will be called haustoria in this paper. The vesicles have been regarded by those using the term as fruiting structures, probably sporanges, a supposition which is supported by Peyronel's tentative account of their germination. The mycelium is nonseptate, at least within the host. In diameter it varies, depending somewhat upon the character of the tissue in which it grows. The first strands which enter the root are 6 to 11 μ in diameter, whereas those which develop later sometimes measure 12 to 13 μ. The hyphal walls are conspicuously thick whether the strands are inside or outside the host tissue. Within the tissue intercellular strands often show small pointed projections extending between the rounded corners of the cells at their junction, suggesting a plasticity of the fungus wall in early development (fig. 2). The comparative thickness of the wall, and the somewhat angular molding of contour at intervals are the more distinctive mycelial characters. The septa, if such they may be called, which are found in mycelium outside the host are usually curved, and appear to be derived from an interior lining of the wall rather than from the thick outer wall.

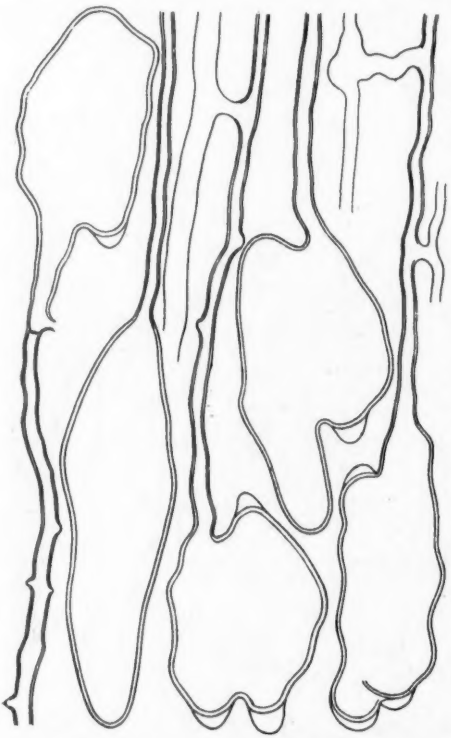


FIG. 2.—"Vesicles" of the mycorrhizal fungus from roots of *Melilotus alba*, showing characteristic shapes which develop from the pressure of surrounding cells.

The haustoria present a complete series of forms from the simplest protuberance of the mycelial wall to branched structures of great complexity. They rarely develop in the outer cells of the cortex in which the mycelium passes readily through cells. In deeper cells haustoria may appear as small protuberances or as thick, blunt projections on intercellular strands. In deeper tissue, which appears to have a richer protoplasmic content, haustoria branch abundantly, producing at the ends of thin hyphal branches a much-lobed, very delicate structure, seemingly without wall or membrane, and often filling a large part of the lumen of the cell (fig. 3). This terminal structure has been interpreted as the product of the digestion of the fungus by the host, and beyond doubt it does disintegrate very soon into an amorphous mass. Whether the haustoria are digested by the tissue which they enter or whether they disintegrate after a brief period of service to the fungus requires further study.

The vesicles are terminal, and usually intercellular in position in the outer cortex, where the mycelium produces but few haustoria. The number produced varies greatly in different roots of the same plant and also in different species. They are usually few in alfalfa and very abundant in sweet clover. In shape they are typically ovate with a slight papilliform projection at the distal end, but pressure from the surrounding cells forces them into a great variety of distorted forms (fig. 1). They vary in length from 75 to 150 μ and in width from 25 to 65 μ. They appear to be separated from the hyphae by merely a thickened portion of the membrane which surrounds the contents of the vesicle. The first vesicles formed in a root usually contain a few large oil globules, and appear imperfectly developed. Occasionally, when the cortical tissue begins to disintegrate, great numbers, which appear filled with small oil globules of uniform size, are formed.

PENETRATION OF THE ROOT BY THE FUNGUS

When small newly infected rootlets are crushed under a cover glass and examined under the microscope, fungus hyphae are often seen passing along the epidermis and entering the root, where the characteristic haustoria develop. The course of penetration has also been traced in stained sections from material imbedded in paraffin. One of these is shown in Plate 1. Figure 3 was drawn from a similar penetration found in a razor section of a sweet-pea root, and shows the characteristic appearance of the fungus in its advance to the deeper cells of the cortex. The hyphal strands outside the root are always empty at this stage. In this instance one of the two entering hyphal branches is greatly swollen in the epidermal cell, producing a structure like an appressorium. The mycelium in the outer cells is empty, and is contorted in a way suggesting that its passage from cell to cell is difficult.

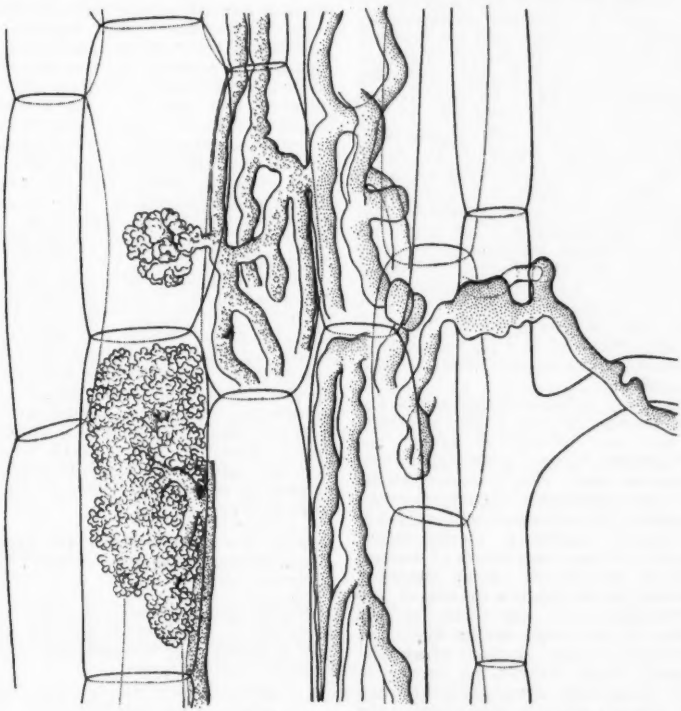


FIG. 3.—Penetration of a root of *Lathyrus odoratus* by the mycorrhizal fungus.

The intercellular strands which produce haustoria have abundant contents. No hyphae filled with protoplasm have been found connecting the mycelium in the root with that outside.

GROWTH OF THE FUNGUS IN CULTURE MEDIA

As soon as the fungus was distinguished by its morphological character in the plant, efforts were made to isolate it in pure culture by the usual laboratory methods. It was found that in the smaller rootlets of alfalfa and clover the fungus was followed so closely by soil-inhabiting fungi and by bacteria that the apparent beginnings of growth of the mycorrhizal fungus were soon submerged and lost. In such rootlets surface sterilization sufficient to destroy the adherent soil organisms always seemed to penetrate the entire cortex. Roots of plants having a thicker cortex, as sweet pea and onion, were then chosen for isolation, and plants were grown in a soil held at a low temperature presumably unfavorable for the troublesome Fusarium species often found in the outer cells. Even in plants thus grown, fungi often grew out from root cortex that appeared uninjured, and bacteria always appeared whenever any fungus growth occurred.

In these isolation experiments the only culture media which appeared to give any growth at all of the mycorrhizal fungus were agar alone or prune agar. When razor sections of roots containing mycelium with abundant protoplasmic contents were thoroughly washed in sterile water, stripped of the outer layers of cortical cells by the careful use of dissecting needles under the low power of the microscope, and placed in poured plates of clear agar before they had solidified, fungus strands which developed could usually be traced to their origin by observation through the bottom of the plate with the microscope. The only fungus strands in such plates which were traced to the mycelium with haustoria in the host cells so clearly that their origin seemed indubitable were of a distinctive character, differentiating them clearly from most fungi in such cultures. These fungus strands were of large diameter, like those in the host tissue, narrowing to a blunt point at the end, and were thrust into the agar almost as straight as stiff bristles, rarely showing a flexuous course or having branches. On the third or fourth day after the plate was made the growth sometimes attained the length of 3 to 4 mm. After this the granular protoplasmic contents began to recede, leaving a series of thin membranous septa. The recession of the protoplasm was usually contemporaneous with the appearance of great numbers of bacteria in the root tissue and its rapid destruction. The agar seemed unable to support growth of the fungus when the host tissue failed. When the longer strands were severed from the host tissue with a sterile needle the protoplasmic contents oozed from the cut ends, and growth ceased. After observation of growth from thin sections seemed to establish the character of the mycorrhizal fungus developing in these culture media, many segments of invaded roots were given surface sterilization with bichloride of mercury and placed in agar, some liquid media, and sterile soil; but in every occasional instance where fungus growth was detected a bacterial growth also occurred, destroying the plant tissue and submerging the fungus. Thus no method of separating the mycorrhizal fungus from its bacterial associates was found, nor did any of the culture media tried appear capable of supporting the fungus growth apart from the host tissue. The fact that strands of the fungus can be found loosely attached to the roots has been regarded as indicating its ability to develop outside of plant tissue, and presumably on artificial substrata; but it is not impossible that this appearance is deceptive, and that the fungus is in fact an obligate parasite capable of growing only in or closely attached to root cortex.

SPECIES OF LEGUMINOSAE IN WHICH THE MYCORRHIZAL FUNGUS HAS BEEN FOUND

During the summer of 1923 the roots of all easily accessible legumes were examined. The fungus was found in the following plants:

Falcata comosa (L.) Kuntze (*Amphicarpa monoica* Ell.).
Astragalus parviflorus (Pursh) MacM.
Lotus americanus (Nutt.) Bisch. (*Hosackia americana* Piper).
Lathyrus odoratus L.
Lathyrus tingitanus L.
Medicago sativa L.
Melilotus alba Desr.
Melilotus officinalis (L.) Lam.
Petalostemum purpureum (Vent.) Rydb.
Phaseolus vulgaris L.
Soja max (L.) Piper.
Trifolium pratense L.
Trifolium hybridum L.
Trifolium repens L.
Vicia sparsifolia Nutt.

There was great variation in the extent to which the root systems were invaded. Roots with a relatively thick cortex, as of peas and clover, generally showed more abundant development of the fungus and a more extensive invasion than beans which have a thinner cortex. There appears to be a difference in infestation between closely related species, the roots of which can hardly be distinguished morphologically. Thus red clover appears to be more thoroughly infested than alsike or white clover.

The fungus was not found in roots of *Oxytropis* sp., *Cicer arietinum* L., or *Lupinus perennis* L. There is no apparent explanation for the complete absence of the fungus from these three species.

DISTRIBUTION OF THE FUNGUS IN AGRICULTURAL LAND

The territory in which the mycorrhizal fungus was first found in native as well as in introduced legumes is comparatively old agricultural land, on the greater part of which leguminous crops have been grown repeatedly. In these older districts many growers believe, not without some good evidence, that certain legumes, especially clover, do not flourish as when first introduced. This opinion is supported by the fact that clover, at least, is at present more successful in newly cleared land. Alfalfa also is not generally so successful in the humid eastern States as in regions of lesser rainfall or under irrigation. These facts suggest the question whether there is any correlation between the abundance of this mycorrhizal fungus and the vigor of these plants. Perhaps newly cleared forest land in which leguminous plants were not numerous and semiarid land with a small plant population of any kind are when first cultivated less thoroughly infested or are free from this fungus.

These questions were in mind when in the summer of 1923 an opportunity was afforded to examine alfalfa and peas in Utah, Idaho, and Montana and also on newly cleared land in Michigan and Wisconsin. It would serve no useful purpose to list here the localities from which specimen plants were taken, or to describe the different cultural conditions under which the plants were grown. It suffices to state that no field however recently reclaimed in arid regions was found free from infestation, and but few mature plants were uninvaded. On newly cleared land in one locality in northern Wisconsin where peas were the first crop, the fungus was found only in plants growing in a few low spots, though at the time examination was made the plants were so young that invasion to a conspicuous degree might not yet have occurred even if the fungus were present. In Montana the fungus was found in the roots of native species on a mountain side far above cultivated fields. Plants in reclaimed swamps in Wisconsin have whiter roots than those in surrounding fields, and indeed in the spring clover has been found in such locations in which no root infestation was discovered. However, a later search in the summer usually disclosed some invasion, though not with accompanying discoloration. It thus appears that not many leguminous plants which are potential hosts reach maturity anywhere in the United States without becoming invaded; and conspicuous vigor in these plants in any locality can hardly be ascribed to the absence of this fungus. On the other hand, it is not easy to determine quantitative differences in invasion in different soils, and much work must be done before such differences will be demonstrated, if they exist, and their effect upon the development of the plants determined.

RELATION OF THE SEASONAL DEVELOPMENT OF THE PLANT TO THE FUNGUS INVASION OF THE ROOTS

During the autumn of 1922 and the entire following year roots of clover and alfalfa were examined from time to time at Madison, Wis., to determine approximately the time at which smaller roots of these plants are developed and when they are invaded by the mycorrhizal fungus. Agronomic and botanical literature appears to contain little information regarding the relation of top growth to root growth in our perennial and biennial legumes, and no information regarding the longevity of the smaller roots. About the middle of April, 1923, as soon as the ground was free from frost, many clover rootlets washed from the soil were found yellow in color. Although the discoloration at this time was largely in the cell walls and had no relation to the presence of the fungus, many of the small roots from all plants in established clover fields contained the fungus, though it was sometimes absent from volunteer plants in fertile gardens. By May 22 the new growth of alfalfa and sweet-clover roots, though varying greatly in different locations, usually amounted to between 1 and 2 inches. In the basal part of this new

growth and extending through about a third of its length the fungus was usually found, apparently advancing from the old root. Plants in soil too dry for vigorous growth showed more invasion than those in more favorable locations. The dry weather, which was also retarding the growth of clover, continued and one week later roots from the most vigorous field were invaded to within an inch of the end, while those from fields a little less vigorous showed in half the roots invasion extending almost to the tip. Soon after this the fungus was found advanced nearly or quite to the root tip in almost all roots, and later in midsummer great numbers of rootlets in the upper foot of soil died. The factors responsible for the death of these roots can hardly be distinguished and evaluated from the evidence at hand. The dryness of the soil alone certainly must have contributed, but it appears also that the invaded rootlets were less resistant to drought, and it may be that the mycorrhizal fungus advanced so far into the growing point of many of them as to cause death. In any case, this loss of roots, as observed during two summers, has left the clover plant with a greatly reduced root system during midsummer. Alfalfa suffers quite as heavily, and during July and August has a surprisingly meager number of living root ends possessing root hairs which can absorb water in the upper 8 inches of soil. Deeper roots do not suffer so great a loss; and in fact the mycorrhizal fungus is less abundant at lower levels, being absent in one instance at a depth of 3 feet. The roots of the plant examined in the instance cited continued to an undetermined depth in very hard subsoil, but there were comparatively few rootlets below the region within which the fungus was abundant.

When rainfall came in late summer and autumn and root development was resumed, longer and longer root ends were free from the fungus. On September 24, the vigorous new roots of red clover near the surface of the soil had 1 or 2 inches of clean growth, while the deeper roots which had grown less rapidly were still invaded almost to their ends. From this time on root growth progressed more rapidly than the fungus invasion. In garden peas, which mature in early or midsummer, there is a steady increase in the proportion of root system invaded until the plant is mature. In the sweet pea, an annual which requires a much longer period of time for its maturity, the root system is found to be thoroughly invaded in mid-July. Little root growth seems to take place thereafter, and the root system slowly dies off during the latter part of the life of the plant.

From this brief outline of the conditions observed in the field it appears that the extent to which roots are found invaded at any time depends largely upon the rapidity with which roots have been growing previously, and presumably also on a temperature favorable to growth of the fungus. Diminished growth of roots at high soil temperatures apparently leads to a complete invasion of the root system. Unfortunately, no study of the rate of root growth of perennial legumes during the season has been made. Therefore much work will be required to determine to what extent root invasion at any time is due to retarded root growth and to what extent it is due to fungus growth.

Some experimental work has been done to determine the range of soil temperature within which the fungus will enter roots. In a preliminary trial in the winter of 1922, it was found that the fungus entered clover roots abundantly at soil temperatures ranging from 16° to 30° C. Another experiment was started in November, 1923, to determine how far beyond this range infection occurs. In this experiment soil was maintained at temperatures of 33°, 15°, 12°, and 9° C. The soil used was loam from woodland, mixed with a small amount of sand containing mycorrhizal-infested sweet-clover roots. Peas and sweet peas were grown in the sand alone. The moisture content of the soil was not determined, but it was ample for good growth, and was maintained uniformly during the experiment. Garden peas, sweet peas, and the taproots of young sweet-clover plants were planted on November 17. Growth of all of these plants was exceedingly slow at 9° C. On December 29, roots of peas at 15° were found extensively invaded, the plants at that time having formed the fifth true node, but no infection was found at 12°. On January 11, peas and sweet peas at 33° had small portions of rootlets invaded, but none of the few branches produced by the Melilotus taproots showed invasion. On January 15 some invasion of sweet pea and Melilotus roots was found at 12°, and finally on February 9, one sweet-pea plant grown at 9° was found abundantly infested. Thus root invasion can take place within the entire range of temperature at which these plants are able to make much growth.

SIGNIFICANCE OF THE MYCORRHIZAL FUNGUS IN LEGUMES

The relation between the fungus and its host plants described in these pages appears to be a long-standing case of mutual adaptation to which the term "symbiosis" may appropriately be applied; but here there is no apparent benefit accruing to the plant invaded. The discoloration and eventual disintegration of the cortex of the roots and the slow growth of the plants at the time when root invasion reaches its maximum suggest that the plant is retarded and injured, at least in so far as vegetative growth is concerned. This may not be determined until the fungus is secured in pure culture. But some evidence of apparent value has already been obtained. This fungus can be destroyed with others in the soil by sterilizing agents, and legumes grown in soil so treated seem to thrive better than in untreated soil. In the spring of 1922, alfalfa, sweet peas, and red, white, and alsike clovers were grown in a small plat which had been treated with formaldehyde the previous autumn, and had been kept covered during the winter to prevent reinfestation. A similar plat was soaked with water to which no formaldehyde had been added. At examinations made during the season, root growth appeared to be about equal for all plants in both plats. But the roots in the treated soil remained white and clean until late in the summer, when a little mycorrhizal infestation began to appear. No other root parasite was detected on plants in either plat. On July 16 the sweet-pea plants in treated soil were twice as tall as in the untreated soil, and had twice as many blossoms. Unfortunately, mosaic appeared in all plants at this time, whereupon blossoming ceased; but the plants in the treated plat remained green throughout the season, while those in untreated soil died and shriveled. The clovers and alfalfa showed little difference until July 30, when the plants in the treated plat stood 1 to 2 inches taller, a superiority which was maintained throughout the autumn. These results agree with Demeter's (*1*) work with *Vinca minor*, but like his are open to the objections that other unrecognized soil parasites may also have been excluded by treatment, and that soil changes favorable to the plant may have resulted from the treatment. Work is now in progress which may furnish more adequate evidence of effect of this fungus on its host plants.

99186—25†——4

MYCORRHIZA OF HERBACEOUS PLANTS OTHER THAN LEGUMES

In order to determine whether roots of plants other than Leguminosæ are subject to this type of mycorrhizal invasion, a number of cultivated plants and weeds growing at Madison, Wis., have been examined. A similar condition has been found in the following plants:

Allium cepa L.
Asparagus officinalis L.
Aster spp.
Bidens vulgata Greene.
Eupatorium perfoliatum L.
Frageria virginiana Duch.
Ipomoea purpurea (L.) Roth.
Panicun capillare L.
Leontodon taraxacum L. (*Taraxacum officinale* Web.)
Verbascum thapsus L.
Viola sp.
Zea mays L.

A mycorrhizal fungus was not found in a considerable number of other species which are not listed here since the number of plants examined was usually small. It is of interest to record that with the exception of an intensive invasion of rootlets of *Solanum tuberosum* growing in sandy soil in a greenhouse, the author failed to find any mycorrhizal fungus of this type in such solanaceous plants as he has had occasion to examine, this failure being in general agreement with the findings of Magrou (*4*) in France.

None of the plants listed above have been examined in sufficient numbers to indicate clearly whether the fungus is constantly associated with their roots or not. In Panicum and Viola especially, its comparative scarcity in the plants examined suggests that it is not a constant associate. Some varieties of sweet corn seemed more thoroughly infested than other varieties of *Zea mays*, half the small roots in one field being invaded, whereas on the same date (Sept. 1) not more than a quarter of the roots of a dent variety growing close by were found invaded. Roots of strawberry plants from several gardens showed wide differences in extent of invasion. Plants of very vigorous growth from one garden had nearly all rootlets filled almost to the end, while those from a neighboring garden had but few infected rootlets.

Whether the fungus in this wide range of plants belongs to the same or to several species, or to specialized races can not be determined readily until the fungus has been obtained in

pure culture. It may be of interest to add, however, that when onion sets were planted with the legumes in the soil temperature series described previously, their roots became about equally (though more slowly) invaded except in soil at 9° C. This fact may indicate that the same fungus entered both plants, since the soil used was taken from a locality where no species of Allium has been found.

SUMMARY

1. During a study of the fungus parasites of the roots of peas and other legumes it has been found that the roots of nearly all our common leguminous crops, wherever grown, are extensively invaded by a characteristic fungus which has previously been known in a considerable number of plants as a mycorrhizal fungus.

2. The term mycorrhizal fungus as used here does not carry with it any implication that the association of the fungus with the higher plant is of benefit to the latter plant. In fact, there is some evidence which indicates that it is more or less injurious.

3. So abundant is this mycorrhizal fungus that it appears unlikely that many plants of alfalfa, clover, peas, and other legumes ever reach maturity without having their roots more or less invaded.

4. The fungus is found only in the primary cortex.

5. The mycelium is coarse, nonseptate in the roots, and though it passes through the outer cells when entering the root is chiefly intercellular in the deeper cells, advancing in long strands toward the growing end. It sends into the deeper cells haustoria which are often of complicated structure, filling more than half the lumen of the cell. The contents of many of the cells containing haustoria become greenish yellow, thus imparting a characteristic discoloration to the entire root. The taxonomic position of the fungus has not been determined, but it appears to belong among the Phycomycetes.

6. No culture medium has yet been found which appears to be capable of supporting the growth of this fungus independently of host tissue.

7. The new growth of perennial clover and alfalfa roots in early spring contains but little of this fungus, invasion occurring at least in part from the growth of the previous season. When root growth is retarded in midsummer, the rootlets become invaded almost to their tips. In autumn, when root growth is again rapid, the fungus is unable to keep pace with it and the new growth is largely free from invasion.

8. The fungus has been found able to invade roots of peas and sweet peas which are grown in a constant soil temperature as high as 33° and as low as 9° C. These temperatures approach closely the extreme limits at which these plants are able to grow.

9. In a single plat experiment clover, alfalfa, and sweet peas were found to grow more vigorously in soil in which this fungus had been killed by formaldehyde, but it is not certain that the absence of this parasite is the only factor which was responsible for the benefit.

10. A list of plants other than legumes in which this same type of mycorrhizal invasion has been found is given.

LITERATURE CITED

(1) DEMETER, K.
1923. UEBER "PLASMOTYSEN"—MYRKORRHIZA. Flora 116: 405–456, illus.

(2) JANSE, J. M.
1897. LES ENDOPHYTES RADICAUX DE QUELQUES PLANTES JAVANAISES. Ann. Jard. Bot. Buitenzorg 14: 53–201, illus.

(3) JONES, F. R.
1923. MYCORRHIZAL FUNGI IN THE ROOTS OF LEGUMES. Rpt. Internat. Conf. Phytopath. and Econ. Ent. Holland 1923: 204–205.

(4) MAGROU, J.
1921. SYMBIOSE ET TUBÉRISATION. Ann. Sci. Nat. Bot. (X) 3: 181–296, illus.

(5) PEYRONEL, B.
1923. FRUCTIFICATION DE L'ENDOPHYTE À ARBUSCULES ET À VÉSICULES DES MYCORHIZES ENTOTROPHES. Bul. Soc. Mycol. France 39:1 19–126, illus.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, $1.00 PER YEAR (DOMESTIC)
$5.25 PER YEAR (FOREIGN)

▽